RECONSTRUCTION OF THE THORACIC OESOPHAGUS WITH INTESTINAL AUTOTRANSPLANT, USING MICROVASCULAR ANASTOMOSIS TO THE AORTA

An experimental study of technique and tissue changes

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I. MALMFORS, G. The microvascular anastomosis to aorta - An experimental study in the piglet. Int J Microsurg. Accepted for publication.

II. MALMFORS, G., HOLMIN, T. and OKMIAN, L. Reconstruction of the thoracic oesophagus using autotransplanted small intestine - An experimental study in the piglet. Scand J Thor Cardiovasc Surg. Accepted for publication.

III. MALMFORS, G., LEANDER, S., BRODIN, E., HÅKANSON, R., HOLMIN, T. and SUNDLER, F. Peptide containing neurons intrinsic to the gut wall - An experimental study in the pig. Cell Tissue Res 214: 225, 1981.

IV. MALMFORS, G., HÅKANSON, R. and SUNDLER, F. Jejunal autotransplantation: Effects on mucosal architecture and endocrine cells - An experimental study in the piglet. Submitted to Gut.

Reference to these papers will be made with the numerals I - IV.

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INTRODUCTION

In about 10 per cent of newborn babies with oesophageal atresia the distance between the oesophageal segments is too long for a primary anastomosis to be performed. There are two alternative ways to bridge the gap; a procedure that elongates the available oesophagus or a procedure in which the gap is bridged with a substitute.

In adult surgery several different methods to substitute the oesophagus have been tried both clinically and experimentally ever since BIRSCHER 1894 performed oesophageal reconstruction with a skin tube. Substitutions have also been performed using stomach, jejunum, colon, blood vessels, inert tubes etc.

Within pediatric surgery, LUNDBLAD in 1921 used colon for oesophageal reconstruction in a three year old child with corrosive oesophagitis. He demonstrated that the substitute grew with the child and functioned satisfactorily 15 years after the operation. SANDBLOM 1948 was the first to use colon for oesophageal reconstruction in oesophageal atresia. WATERSTON and SHERMAN (1957) developed reliable methods to use the colon for oesophageal substitution and reported a high survival rate in a large number of patients. The common denominator for these substitution methods is that the interposed viscus receives its vascular supply via a vascular pedicle from the abdomen. Autotransplantation of an intestinal segment revascularized via intrathoracic vascular anastomoses has not yet been performed.

All substitution methods tried clinically have disadvantages, specific for different methods. The development of operative methods has therefore followed alternative paths. HOWARD and MEYERS (1965) reported a technique to elongate the upper oesophageal segment by bouginage. Hemicircular incision of the oesophageal pouch was reported in 1969 by OKMIAN and LIVADITIS. Circular cervical myotomy to bridge the gap was developed and described by LIVADITIS and OKMIAN (1969), LIVADITIS, RÄDBERG and ODENSJÖ (1972) and KORNFÄLT (1973). REHBEIN (1971) described a method to elongate the segments by means of a fistula between the oesophageal segments.

Autotransplantation of intestine into the neck of a dog was performed by CARELL as early as 1907. SEIDENBERG (1959) was the first to perform a successful autotransplantation of an intestinal segment to repair a cervical oesophageal defect in man. The basic principles of the modern microsurgical technique were presented by JACOBSSON and SOAREZ (1961). This technique and the microsurgical instruments and sutures have since then undergone an evolution. A modern technique for microvascular anastomoses in rapidly growing young animals was described by OKMIAN (1976). MEEUWIS (1980) reported a series of puppies operated with intestinal autotransplantation as a substitute for thoracic oesophageal defects. Thus, prerequisites exist to use revascularized intestinal autotransplants to substitute the oesophageal defect in newborns with long-gap oesophageal atresia. As a step in the development of oesophageal substitution after resection for malignancy MEIER (1968), GÖBER et al (1972), KOZUSCHEK et al (1972), DRAGOJEVIC et al (1975) and ODHIAMBO (1979) tried intrathoracic autotransplantation of intestinal segments in dogs. Their results were not encouraging, but highlighted the special problems associated with this approach. One major problem was the difficulty to avoid kinking of the relatively long vascular pedicle when the anastomoses were performed to intercostal or internal thoracic vessels. The use of a method where the transplant artery is anastomosed to the aorta offers freedom to choose the place for the anastomosis and results in a shorter vascular pedicle, thereby reducing the risk of kinking.

AIM OF THE STUDY

The aim of the study was to develop a method to microanastomose small arteries to the aorta (I) and to use this method in revascularization of an intestinal segment to be used as an oesophageal substitute (II).

Contrary to the interposition methods in clinical use, autotransplantation implies a total extrinsic denervation of the intestinal transplant. Intestinal functions (motor activity, blood flow, secretion and absorption) are controlled by a complex interplay between neuronal and hormonal signals. The former are exerted by intrinsic and extrinsic autonomic nerves; the latter by the diverse systems of gut endocrine cells.

Thus, another aim of the study was to examine the transplants with respect to the autonomic nervous system and the motor activity (III), the mucosal architecture and the endocrine cell populations (IV). This knowledge is essential for the proper assessment of the virtues of a clinical application.

MATERIAL AND METHODS

Seventy-two piglets of Swedish Landbreed weighing 4.4 - 9.0 kg (mean 6.5 kg) were operated. Animals that were obviously undernourished or unhealthy were not used; otherwise, no selection was made.

The major part of the basic studies of the technique to microanastomose arteries to the aorta was performed intraabdominally (I) for practical reasons. The experience gained was used in the intrathoracic application (II), where revascularized intestinal autotransplants were used for oesophageal reconstruction. A method to perform autotransplantation of jejunal segments without producing ischaemia in the intestinal wall was developed. This implied a possibility to differentiate between the effects of the extrinsic denervation on the one hand and the effects of ischaemia and exposure to perfusion fluid on the other (III, IV). In order to evaluate the results obtained from the morphological investigations, vagotomies were performed to clarify the origin, distribution and functional influence of the extra- and intramural nervous systems (III). Thus, four different operative procedures were used.

OPERATIVE PROCEDURES

In all cases, anaesthesia was induced with Ketamine hydrochloride (Ketaminum NFN) and Droperidole (Droperidolum INN) i.m. After venous cannulation and endotracheal intubation, the animals were artificially ventilated with equal parts O_2 and N_2O. Alcuronium chloride (Alcuronum NFN) was used for muscular relaxation.

The operations were performed under sterila conditions, using instruments adapted for pediatric surgery and microsurgery. During the performance of microvascular anastomoses, either an operative microscope

Zeiss OPMI 1 or magnification glasses (2.5 x) were used. The four different operative procedures used were:

- Abdominal intestinal autotransplantation. The transplant artery was anastomosed to the aorta and the vein to the inferior vena cava (I, III and IV)
- Intrathoracic intestinal autotransplantation as a substitute for part of the oesophagus (I, II)
- Autotransplantation without ischaemia (III, IV)
- Truncal vagotomy (III)

Abdominal intestinal autotransplantation

The series comprised 15 animals. A jejunal segment suitable for transplantation was identified through a midline incision. The aim was to find an artery approximately 1.5 mm in diameter and a corresponding vein 2 - 3 mm in diameter, since this size corresponds to that expected in a clinical application in infants. The transplants were taken from the proximal half of the jejunum. A wedge-shaped resection of approximately 10 cm of the intestine was performed. Adjacent segments of the intestine became ischaemic, thereby requiring additional resection. The total resected length ranged from 0.5 to 1 m. Intestinal continuity was restored with an end-to-end suture in two layers with Dexon^{R} 6/0. The abdominal aorta and the caval vein were dissected free several cm proximally from their bifurcations. The vascular pedicle was divided as proximal as possible. A 1 mm in diameter siliconized rubber catheter was inserted into the artery, and perfusion with refrigerated dextran-electrolyte solution (Perfadex^{R}) with heparin 5 IE per ml was started immediately (Fig. 1, page 38). The transplant vein was anastomosed end-to-side to the caval vein using a continuous 9/0 silk or Ethilon^{R} suture.

The microvascular anastomosis to the aorta

The aorta was partially clamped with a Potts U-shaped vascular clamp. A suitable oval opening in the aortic wall was made with a scalpel, scissors, punch or, in the latest 6 cases, specially-constructed vascular scissors. These have spiral-shaped blades and cut an oval opening with sharp, even edges through the entire aortic wall (I) (Fig. 2 A, B and 3, page 40 and 41). The transplant artery was cut obliquely and anastomosed to the aorta with a continuous 8/0 - 9/0

silk or Ethilon^{R} suture. Pulsative bleeding from the edges of the transplant was the criterion of successful revascularization, provided there were no signs of venous obstruction. The proximal end of the autotransplant was closed, and the distal end was drawn through and sewn to the abdominal wall as a jejunostomy. The period of ischaemia varied between 90 and 120 minutes.

Intrathoracic intestinal autotransplantation as a substitute for part of the oesophagus

The series comprised 32 animals. The transplant was taken from the proximal jejunum in 30 cases and from the lower jejunum in 2 cases, and perfusion was started as described above. A thoracotomy was performed in the left fifth intercostal space. The first part of the descending aorta, the last part of the hemiazygos vein and the central part of the thoracic oesophagus were dissected free. A venous anastomosis was performed with microsurgical technique between the transplant vein and the hemiazygos vein. In most cases an end-to-end suture was possible, but in some cases an end-to-side anastomosis was preferred, due to a big difference in width between the two vessels. The perfusion was interrupted, and a microvascular anastomosis was performed between the transplant artery and the aorta, using the method described. The specially constructed vascular scissors were used in the last part of the series. After successful revascularization, approx. 5 cm of the thoracic oesophagus was resected and the autotransplant was trimmed to the appropriate length to fit in between the oesophageal segments (Fig. 2, page 56). The anastomoses between the oesophagus and the transplant were sewn in one layer with interrupted sutures of 6/0 silk. The vascular pedicle was usually longer than the distance between the aorta and the oesophagus, thereby creating a risk of kinking. However, great effort was made to avoid it. Finally, the thorax and the abdomen were closed. The time of ischaemia varied between 80 and 130 minutes.

Autotransplantation without ischaemia

The series comprised 17 animals. Suitable mesenterial vessels were exposed in the same way as was described above. The vein was divided between microvascular clamps, and immediately reconstructed with a continuous 9/0 - 10/0 Ethilon^{R} suture. The artery was divided and re-

sutured in the same way. During the vascular clamping, the circulation of the intestine was maintained via arcade vessels. A wedge resection of 10 - 20 cm of jejunum and its mesentery was performed. Thus, an autotransplant was obtained that was already revascularized (Fig. 1 A - C, page 69). This autotransplant had not been subjected to ischaemia or to perfusion. The adjacent parts of the intestine became ischaemic and required resection. In 6 cases the transplant was sewn to the abdominal wall as a jejunostomy, and the intestinal continuity was restored end-to-end with Dexon^{R} sutures. In 11 cases the transplant was sewn back into intestinal continuity.

Truncal vagotomy

Nine animals were subjected to vagotomy. In 5 cases this was performed intraabdominally. One of these piglets had earlier been transplanted, and was vagotomized one hour before sacrifice. In 2 cases the vagotomy was performed cervically, and in 2 cases intrathoracically.

Postoperative regimen

I.v. perfusion with dextran 70 6% 50 ml per kg b.w. and 500 ml Ringer-glucose solution was started during the operation. Three or four days postoperatively 100 ml Ringer-glucose per kg b.w. was administered i.v. The enteral feeding varied. The piglets operated intraabdominally were allowed to take fluid from the second day postoperatively. The piglets operated intrathoracically had transanastomotic tubes or gastrostomies; the latest 10 animals were allowed to drink freely. During the operation 5 million IE depot penicillin and 0.5 g Cephalotine^{R} were given. Cephalotine was continued for three or four days postoperatively.

Follow up

The piglets operated with intraabdominal autotransplantation with vascular anastomosis to the aorta were followed between 3 and 17 weeks. The piglets operated intrathoracically with oesophageal substitution were followed up till 5 weeks. The animals operated with abdominal autotransplantation without ischaemia were followed between 6 and 8 weeks, while the vagotomized animals were followed between 1 hour and 2 weeks.

The animals that died postoperatively were subjected to an autopsy, except for a few cases where autopsy was not possible for technical reasons. The animals that died during the operation were not examined further.

At sacrifice, the transplant, the vascular pedicle and adjacent parts of the anastomosed vessels were dissected free. Before the pig was killed, specimens were taken from the wall of the autotransplant for histologic or chemical analyses and for studies on motor activity. Control specimens were taken from the jejunum in situ close to the intestinal anastomosis. Vascular anastomoses were calibrated with catheters of different dimensions before they were transected and macrophotographed. Anastomoses between the transplant and oesophagus were incised and photographed. In selected cases, specimens were taken from vascular anastomoses for histologic examination.

RADIOLOGICAL METHODS

Barium swallow

A barium swallow was performed on the animals that survived more than two weeks after intrathoracic autotransplantation. The contrast was administered through a catheter in the proximal oesophagus while the animal was under general anaesthesia. The passage through the transplant was followed by fluoroscopy and documented on X-ray films.

Angiography in vivo

Those animals that had been operated intrathoracically and survived more than two weeks, as well as 4 of the piglets operated intraabdominally with microvascular anastomoses to the aorta and the vena cava, were subjected to in vivo transplant angiography. The examination was performed via the femoral artery while the animal was under general anaesthesia. At first an aortography was performed, and then a selective angiography was attempted.

Angiography of the specimen

In 8 piglets operated with intraabdominal autotransplantation, the vessels of the autotransplant were filled with low-viscous barium contrast (Micropaque^{R}). In 5 of these cases, the special vascular scissors had been used. The contrast was injected with 13 kPa pressure into the ligated aorta. Exposures were taken in perpendicular projections.

HISTOLOGICAL METHODS

Specimens from the autotransplants and from the jejunum in situ were immediately frozen in a mixture of propane and propylene cooled to the temperature of liquid nitrogen. After freeze-drying, the specimens were fixed either by exposure to gaseous formaldehyde for 1 hour at 80°C or to gaseous diethylpyrocarbonate for 3 hours at 55°C and embedded in paraffin in vacou. Sections cut at 6 µm were processed for the immunocytochemical demonstration of the following neurohormonal peptides:

Leu enkephalin	VIP (vasoactive intestinal peptide)
Somatostatin	GIP (gastric inhibitory peptide)
Substance P	CCK (cholecystokinin)
Neurotensin	Motilin
Secretin	

In addition, some specimens were fixed by immersion in cold 4% formaldehyde solution, rinsed overnight in sucrose containing Tyrode buffer and frozen for cryostat sectioning. Sections were cut at 15 µm. These sections were processed for the demonstration of the following neuropeptides: enkephalin, somatostatin, substance P or VIP.

Sections were exposed to peptide antiserum for 3 hours at room temperature (immunofluoroscence) or for 16 hours at 4°C (immunoperoxidase). The site of the antigen-antibody reaction was revealed by fluorescein-isothiocyanate (FITC) -labelled antirabbit IgG (immunofluoroscence) or by unlabelled goat antirabbit IgG, followed by exposure to PAP complex (immunoperoxidase staining). Sections incubated with peptide antiserum inactivated by the addition of antigen in excess served as controls (ALUMETS et al 1979). For the demonstration of adrenergic nerve fibres, sections were cut from formaldehyde treated specimens, mounted in Entellan and examined in a fluoroscence microscope (BJÖRKLUND et al 1972). For the demonstration of acetylcholinesterase the method of KOELLE was used as modified by HOLMSTEDT (1957).

Morphometry

Sections were cut perpendicular to the mucosal surface and stained with Haematoxylin-eosin. The total thickness of the mucosa, the height

of the villi and the depth of the crypts were measured at 125 x magnification. In addition, the number of crypts per unit length were counted. The height of the villi and the depth of the crypts were expressed as percentage of the total mucosal thickness.

Cell counts

For the quantitative analysis of the different endocrine cell populations, immunostained sections cut perpendicular to the mucosal surface were used. The number of each of the endocrine cell types was counted in 4 - 11 randomly selected visual fields in sections from at least four tissue specimens from each animal. (Objective x 10, eye piece x 12.5, visual field diameter 2.8 mm).

CHEMICAL METHOD

The concentration of immunoreactive substance P was determined by radioimmunoassay (NILSSON et al 1977, BRODIN et al 1981).

PROCEDURE FOR STUDYING MECHANICAL ACTIVITY

Muscle strips measuring approx. 20 x 3 mm were mounted vertically on a holder in a 37^{o} organ bath. The free end of the muscle was attached to a lever connected via a spring to a Grass force deplacement transducer for isotonical registration of mechanical activity. The muscle strips were subjected to different stimulation frequencies with or without atropine sulphate, guanetidine sulphate or tetrodotoxin in the bath.

R E S U L T S

OPERATIVE

Abdominal intestinal autotransplantation

Nine of the 15 animals were sacrificed at the time planned. Four more animals survived, but the transplants were necrotic. The reason for the necrosis could not be established. One animal died peroperatively from anaesthesiological complications. Another animal died from a general infection three weeks postoperatively; the transplant was vital at autopsy. In those 6 cases where the special vascular scissors were used for the aortic opening, the transplants survived.

Intrathoracic intestinal autotransplantation as a substitute for part of the oesophagus

The revascularization was successful in 19 of the 32 operations; i.e. there were pulsations in the periphery of the transplant and no signs of venous obstruction were observed. Four piglets survived more than 2 weeks, and were examined with barium swallow and angiography. Ten piglets died from peroperative complications to the anaesthesia given. The microvascular anastomoses failed in 8 cases and the piglets were sacrificed peroperatively. Six animals died from transplant necrosis during the first two postoperative weeks. Three animals died from peroperative haemorrhage, and 1 piglet died from postoperative intestinal obstruction.

One of the surviving piglets died after 5 weeks from a fistula between the cranial oesophageal-transplant anastomosis and the aorta. The transplant was vital. The barium swallow 2 weeks earlier had demonstrated a normal passage through the transplant, but the angiography had failed to demonstrate the transplant vessels. Another piglet was examined after 2 weeks. The angiography demonstrated adequate circulation through the transplant (Fig. 3, page 58), but the barium swallow indicated a possible anastomotic leak. At sacrifice, the transplant was found to be vital (Fig. 4, page 59) and the anastomoses sufficient. Two more piglets were sacrificed after 3 weeks when the angiographies had indicated insufficient circulation. The barium swallow in these cases showed changes in the transplant wall indicating transplant necrosis, which was confirmed at autopsy.

The microvascular anastomosis to the aorta

Thirty-three of the 47 anastomoses performed were initially patent. In 10 of these cases, the animal died peroperatively or in the early postoperative period. In 10 cases transplant necrosis occurred during the first two postoperative weeks. In 2 other cases the transplant was found to be necrotic three weeks postoperatively.

The special vascular scissors were used in 10 operations. The initial revascularization was successful in 9 cases; one piglet died from peroperative haemorrhage. In 6 cases long-term transplant survival was recorded. In one case there was an arterial thrombosis and in another

case the transplant was necrotic without macroscopic thrombosis. One animal died in the immediate postoperative period of hyperthermia.

Angiography in vivo performed in 4 cases operated abdominally and 4 cases operated thoracically demonstrated patent vascular anastomoses in 3 abdominal cases and one thoracic.

Angiography of the specimen was performed in 8 intraabdominal cases; in 5 of which the special instrument had been used (Fig. 4, page 43). A slight narrowing of the first mm of the transplant artery was observed in 5 cases.

At calibration a slight narrowing of the first mm, i.e. that part of the transplant vessel which is situated within the aortic wall, was found in all cases operated intraabdominally.

Macrophotographs of the transected anastomoses demonstrated absence of thrombosis and smooth intimal surface in the anastomotic line (Fig. 5, page 44). The results showed that it is possible to anastomose microarteries (1.5 mm) end-to-side to the aorta with a satisfactory rate of patent anastomoses, if the opening in the aortic wall is cut with the special instrument described.

Autotransplantation without ischaemia

Nine of the 17 animals were sacrificed at the time planned. Two animals died from postoperative intestinal obstruction and diarrhoea respectively; their transplants were vital. Another animal died postoperatively from intraabdominal haemorrhage. Two animals died after 2 to 3 days from transplant necrosis caused by arterial thrombosis, while in 3 cases the transplants were necrotic without macroscopical thrombosis. The 9 surviving animals all had vital transplants surrounded by extensive adhaesions. Of these transplants 5 had been in intestinal continuity, while 4 had been excluded from intestinal continuity.

Truncal vagotomy

The 5 piglets subjected to abdominal truncal vagotomy survived the operation and were sacrificed at the time planned. The 4 piglets that were vagotomized cervically or thoracically died during the immediate postoperative period.

MORPHOLOGICAL, CHEMICAL AND MECHANICAL INVESTIGATIONS

Specimens for histological investigations were obtained from 9 animals operated intraabdominally with an ischaemic period and from 9 animals subjected to autotransplantation without ischaemia. Specimens for studies of mechanical activity were obtained from 4 animals and specimens for determination of the substance P concentration were obtained from 5 animals; all operated intraabdominally with an ischaemic period. From the surviving vagotomized animals, specimens were taken from the vagal stumps and the jejunum.

The nervous system

In the myenteric plexus in the wall of the autotransplants there were numerous nerve fibres and occasional nerve cell bodies displaying VIP, enkephalin, substance P or somatostatin immunoreactivity. Scattered immunoreactive nerve fibres occurred in the smooth muscle. VIP and somatostatin immunoreactive nerves were regularly observed also in the submucosal plexus. Only VIP-containing fibres were numerous in the mucosa. The distribution and frequency of occurrence of the various types of peptidergic nerves were the same in the transplant as in the jejunum in situ. The innervation pattern did not vary with time or degree of ischaemia, nor was it different in transplants included in or excluded from intestinal continuity. The results were independent of whether cryostat or paraffin sections were used. The AchE positive nerves were equally abundant in the wall of the autotransplants and of jejunum in situ. Adrenergic nerves, on the other hand, were absent in the autotransplants, but numerous in all layers of the jejunum in situ (Fig. 3, page 73).

In the vagotomized gut wall the distribution pattern of the different nerves was unchanged. The vagal nerve contained fibres displaying VIP or substance P. Upon ligation of the vagal nerve, the peptide accumulated in swollen fibres on both sides of the vagal ligature, indicating presence in both efferent and afferent neurons. The accumulation was most pronounced above the ligature, i.e. in efferent fibres. Somatostatin and enkephalin-containing nerves were observed only occasionally in the vagal nerve.

Chemical analysis

The determination of substance P in transplants and jejunum in situ

revealed mean concentrations 7.7 pmol/g ± 2.2 (S.E.M., n = 5) versus 6.7 ± 2.0 (S.E.M., n = 5). This is not a statistically significant difference.

Mechanical activity studies

When working against a load, strips of muscle from the autotransplant showed an irregular activity, contrary to the regular activity of the jejunum in situ. The spontaneous activity was not inhibited by tetrodotoxin, a specific blocker of nerve conduction (KAO 1966). Upon electrical field stimulation the transplant muscle and the control muscle responded in a similar way, which was true also after cholinergic and adrenergic blockade. Upon electrical stimulation, the muscle responded with contraction, while in the presence of atropine and guanethidine, the muscle responded with a relaxation. Administration of tetrodotoxin abolished all responses to electrical stimulation, indicating that the responses were neurogenic in nature.

Mucosal architecture

The thickness of the mucosa was unchanged in the transplants which had been in intestinal continuity. Transplants which had been excluded from intestinal continuity had a thinner mucosa. The relative height of the villi was unchanged in all transplants (Table 2, page 88). The villi of the transplants were often slightly club-shaped. The number of crypts per unit length was unaffected by the operative procedures (Table 3, page 91).

Endocrine cells

The intestinal mucosa was found to be rich in peptide hormone producing cells. Enkephalin, GIP, somatostatin and CCK cells were the predominating types. Neurotensin, secretin and motilin cells were few. The enkephalin, somatostatin and motilin cells occurred mainly in the crypts, while the GIP, CCK and neurotensin cells were evenly distributed in the mucosa. The secretin cells occurred almost exclusively on villi. Transplants in intestinal continuity did not differ from the jejunum in situ in number and distribution of endocrine cells. On the other hand, the transplants which had been excluded from intestinal continuity contained a reduced number of certain types of endocrine cells. The reduction of the number of enkephalin, CCK and

somatostatin cells was statistically significant. In the case of GIP cells there was a numerical reduction by 17% which was not statistically significant (Table 4, page 92). The neurotensin, secretin and motilin cells were too few to allow quantitation.

DISCUSSION AND CONCLUSIONS

TECHNICAL CONSIDERATIONS

To autotransplant an intestinal segment to bridge a thoracic oesophageal defect is a complicated and demanding operation for the experimental animal as well as for the operative team, a fact which was reflected in the comparatively high mortality and complication rate. An analysis of the contributing factors is essential for a proper assessment of the results.

Choice of species

The piglet is a suitable experimental animal when effects related to rapid growth are to be studied, since it grows to adult size in half a year. Therefore, this animal is especially suitable for the assessment of pediatric surgical operative methods. Another advantage is the anatomical similarities between the piglet and the infant. However, two anatomical differences were detrimental to the investigation: 1) the piglet lacks an azygos vein, but has a hemiazygos vein; 2) the vascular anatomy of the mesentery is different.

If there were an azygos vein, the operation would have been possible to perform from the right side, which should have been easier as there is more space and the oesophagus is easier to reach. The aorta is more difficult to expose from the right side, but it can be mobilized enough to allow the anastomosis to be performed.

The different distribution of the mesenterial vessels with lack of sufficient arcades meant a loss of intestine in the piglet which should not be necessary when the operation is applied in humans. Furthermore, the extensive intestinal resection resulted in a state of malnutrition, unfavourable for postoperative healing. The piglet also has other disadvantages as an experimental animal. It is sensi-

tive to stress; it makes accordingly great demands on anaesthesia and postoperative care after extensive operations. The porcine vessels are rather friable and the tendency for thrombosis seems to be greater than in man. Even though the dog would probably have been a less problematic species, the great anatomical and biological similarities between the human infant and the piglet indicate that the piglet is a suitable experimental model.

Operative complications

Complications due to the anaesthesia contributed to the mortality in 11 cases. Most of these could have been avoided if an anaesthetist had been available. Haemorrhage is an unavoidable complication in vascular surgery, even if every effort is made to avoid it. The piglets that died of peroperative haemorrhage would probably have survived with blood transfusions. The lack of resources for continuous day and night postoperative supervision meant that complications were not recognized early enough to be dealt with. Several animals that died in the early postoperative period could probably have been saved with more efficient supervision.

The microvascular anastomosis to aorta

A prerequisite for uncomplicated healing in vascular anastomoses is perfect intimal contact. The same is true for microanastomoses independently of diameter or wall thickness. In this study 1.5 mm arteries were anastomosed to the aortic wall, which was between 1.5 and 2 mm in thickness. It was a difficult technical task to obtain an even hole with sharp edges and to obtain perfect intimal contact between the thick aortic wall and the tiny artery. Different methods were tried to provide a suitable opening; scalpel, scissors and punch. The results were not encouraging. The introduction of the spiral-shaped vascular scissors meant a possibility to obtain an even, oval, coniform opening with sharp edges. A larger version of this instrument was found suitable for the aortic opening in surgery for renal artery stenosis by BERGENTZ et al 1978. In the present study, the results with this instrument were satisfactory with respect to transplant survival, frequence of thrombosis and absence of stricture.

The slight narrowing revealed at calibration did not imply insufficient circulation. The narrowing is probably to be regarded as an adaptation

to diminished circulatory demands, since the transplant artery is greatly overdimensioned for the capillary bed of the transplant. Histologic examination of the anastomoses showed normal aortic wall tissue surrounding the intramural part of the transplanted vessel. Of the 8 anastomoses performed with the special vascular scissors only 1 thrombotized. Thus, the results has shown that *it is possible to anastomose microarteries (1.5 mm) end-to-side to the aorta with a satisfactory rate of patent anastomoses* if the opening in the aortic wall is cut with the special instrument described.

The exact intimal adaptation needed for satisfactory results in microvascular surgery was difficult to obtain in the intrathoracic operations, even when the technique developed abdominally was applied. A factor that contributed to the difficulty of obtaining functional arterial anastomoses was the movements of the heart, lungs and aorta. The movements reduced the possibility to use high magnification as the operative area fluctuated from the focal plane. It therefore appeared convenient to use low magnification or magnification glasses, which is a technical disadvantage. The stitches had to be rather coarse because of the fragility of the aortic wall, which also meant a risk of unsatisfactory intimal adaptation.

The problem of thrombosis

Thrombosis is a major problem in vascular microsurgery. In order to avoid it a perfect intimal contact is mandatory. Other important factors are an atraumatic technique, suitable suture material and good haemodynamic conditions in the anastomosis (ACLAND 1973). In a few cases macroscopic thromboses were found, while in several cases transplant necrosis without demonstrable thrombosis existed. It seems probable that the cases of transplant necrosis occurring after a few days were caused by venous microthrombotism, as described by BUSSMAN et al (1972) in ileal autotransplants in dogs. One possible reason for this is a venous dilatation caused by the acute external denervation, which implies a slow venous flow and risk for thrombosis. For prevention of thrombosis, dextran 70 i.v. peroperatively and transplant perfusion with heparinized dextran-electrolyte solution was used. LILLEHEI et al (1964) demonstrated that this perfusion method allows 5 hours of ischaemia without damage to the transplant. Even longer

ischaemic periods can be tolerated, if chlorpromazine is added to the perfusion, or if it takes place under hyperbar oxygenation (FONKALSRUD 1969). In the present study there was no need for such measures. GERMAIN et al (1980) demonstrated that intestinal autotransplantation can be performed without perfusion or cooling of the transplant. Thus, 27 of 30 experimental transplants and 12 of 12 human survived without any perfusion. In the present investigation the method of LILLEHEI was used, as the method of GERMAIN probably makes extreme demands on operative speed.

Clinical aspects

The intrathoracic operations were charged with a high mortality and complication rate. In 4 cases extended survival was recorded, and in another 4 cases the survival was around 1 week. These cases demonstrate that *it is technically possible to substitute part of the thoracic oesophagus with an autotransplanted intestinal segment, revascularized via microanastomoses to the aorta and the hemiazygos vein.*

The results of the experimental substitution operations may seem discouraging, but access to the advanced facilities of intensive medical care would certainly mean a much higher survival rate in man. Among the factors needed for improvement are the possibility to give blood transfusions, resources for continuous postoperative supervision and two operative teams. The results indicate that *the method described* is not ready for clinical use, but *is possible to develop into a clinically useful method for the treatment of oesophageal atresia.* A further development of the method should be experimental at first, and then proceed via operations in adult patients. As the operation is technically demanding, careful preparation and practice is mandatory if it is to be performed with a good safety margin. A possible clinical application in the future should be centralized to a few units to allow accumulation of sufficient experience.

Autotransplantation should be compared with the colonic interposition methods according to SHERMAN and WATERSTON. The autotransplant as well as the interponate is an elastic, vital organ with retained motor function and of approximately the same dimensions as the oesophagus. The function of the cardia is maintained as in WATERSTONS procedure.

But there are two factors that favour the use of autotransplantation. Only the gap between the oesophageal endings has to be bridged, whereas, the colonic interponates have to bridge the distance from the neck to the diaphragma. In autotransplantation only a small intestinal resection is necessary; on the other hand, the colon interposition methods require approximately half the length of the colon.

The main disadvantage with autotransplantation is the risk for thrombosis in the microvascular anastomoses and subsequent transplant necrosis. The colon interposition methods also include a risk of insufficient vascular supply because of the long vulnerable vascular pedicle needed. Autotransplantation in clinical use is probably not much riskier than the colon interposition methods. The recent results of cervical oesophageal substitution with autotransplant favour this view (GERMAIN 1980).

The method described for microanastomosis to the aorta is ready for clinical application. It may be used in homotransplantation of other organs in the newborn period as well as in adults, as soon as the immunological problems are solved. The spiral-shaped scissors are suitable also for other microvascular anastomoses. It has been found useful in the performance of arterio-venous fistulas in the forearm, and it was used experimentally in venous microanastomoses (HOLMIN 1980).

TISSUE STUDIES

A determining difference between autotransplantation and bowel interposition is that the autotransplant is subjected to a total external denervation, exclusion from the lymphatic system, a period of ischaemia and exposure to perfusion fluid. It appears important to gain the most possible information about the reaction of the transplant to these factors.

GOOTT et al (1960) demonstrated that lymphatic regeneration occurs within days and is completed after a few weeks. In the present studies the follow up times were chosen to allow complete lymphatic regeneration. In order to separate the possible effects of ischaemia and perfusion fluid, a method was developed to obtain autotransplants that

were not subjected to either ischaemia or perfusion fluid. The transplants were either excluded from or included in intestinal continuity to give a possibility to separate the effects of intestinal exclusion. A commonly used model for denervation studies is to skeletonize the vessels, thereby dividing most of the extrinsic nerve supply, but leaving the nerve fibres coming along the gut wall and through the mesentery in place. The only operative way to obtain a guaranteed total extrinsic denervation is to divide vessels, mesentery and intestine, as was performed in this study.

The nervous system

There are many reports on intestinal autotransplantation, especially in substitution of the cervical oesophagus (ROBERTS 1961, NAKAYAMA et al 1964, HARRISON 1965, OKMIAN 1976, UEMICHI et al 1977, GERMAIN et al 1980 and others). The reports of the effect of the autotransplantation on the nervous system of the transplant are controversial and surprisingly few. LILLEHEI et al (1962) found no changes in the ganglia in the gut wall, nor did GREEN et al (1966). NAKAYAMA et al (1964), on the other hand, reported changes of the intramural nerve cell bodies with loss of Nissl bodies. COULIC (1972) and MAXIMENKOVA (1972) demonstrated abnormal EMG in experimentally denervated intestines. They discussed the necessity of operative reinnervation in intestinal transplantation. Reports regarding the different types of nerves or the distribution of nerves in autotransplants have not been published.

The gut wall is richly supplied not only with adrenergic and cholinergic nerve fibres but also with peptide containing fibres (FURNESS and COSTA 1980, SCHULTZBERG et al 1980, SUNDLER et al 1980). There is evidence that neuropeptides may act as transmittors, notably substance P, VIP, enkephalin and somatostatin. There are indications of further types of peptides with such properties. The different types of peptidergic (peptide-containing) nerves have their characteristic distribution in the gut wall. The distribution or the frequency of occurrence of the peptidergic nerves was not changed by extrinsic denervation, indicating that these nerves are intrinsic to the gut wall. The fact that the chemical determination of substance P revealed unchanged concentrations after autotransplantation confirmed the immunohistochemical

findings. The cholinergic nerves remainded also unchanged, independently of the operative method. On the other hand, the adrenergic nerve fibres were completely lacking in the transplants, indicating that all adrenergic nerve fibres in the porcine gut wall are extrinsic, contrary to what seems to be the case in certain other species. Around the vagal ligatures VIP and substance P accumulated in swollen nerve fibres indicating that the vagal nerve contains both afferent and efferent peptidergic neurons. As there was no difference in the occurrence of peptidergic nerves after external denervation or vagotomy, the vagal contribution must be quantitatively insignificant. *Thus, the investigation indicates that the major portion of the peptide-containing nerve fibres in the gut is of intramural origin. Autotransplantation causes loss of adrenergic innervation but no other morphological changes in the nervous system.*

Mechanical activity studies

The in vitro study of the motor activity of the muscle in the jejunal wall demonstrated an irregularity in the spontaneous activity of the transplants compared to the regular spontaneous activity of the normally innervated jejunum. The responses to electrical field stimulation and to adrenergic and cholinergic blockade were unchanged after autotransplantation. The response to electrical stimulation after both adrenergic and cholinergic blockade was relaxation, suggesting that *the peptidergic nerves have a relaxing effect on the intestinal smooth muscle*. The results indicate a high degree of autonomy in the intestinal wall and might suggest that external nerve supply has a synchronizing effect; maybe the type of synchronization that according to SZURSZEWSKI (1968) and CODE (1979) is characteristic of "the interdigestive house-keeper".

Mucosal architecture

Histological examination of routinely stained mucosal biopsies from autotransplants has been performed previously, and the reports are controversial. BALLINGER et al (1962) reported mucosal changes including a total loss of villi initially after intestinal autotransplantation in dogs. The mucosa was found to regenerate after a couple of weeks, but the villi remained short and club-shaped. They concluded that the external denervation per se caused the changes. PULL TER GUNNE

(1975) described a slight shortening of the villi after autotransplantation of ileum in dogs. LILLEHEI et al (1962), on the other hand, found no mucosal changes in their pioneering experiments in dogs. ALICAN et al (1971) also described normal mucosa at different times after intestinal autotransplantation in dogs. Furthermore, there are several reports on intestinal autotransplantation in man where normal mucosal morphology has been described (NAKAYAMA 1964, UEMICHI 1977, GERMAIN 1980 and others). In all these reports standard histological staining methods have been used, and the thickness of the mucosa has been subjectively assessed.

In this report a morphometric technique, where the mucosal thickness was measured in perpendicular sections, was used. *The results demonstrated that the denervation per se meant no change of the mucosal thickness or of the relative height of the villi.* The ischaemia or the exposure to perfusion fluid did not seem to influence the mucosal architecture; nor did the follow up time. *The autotransplants that had been excluded from intestinal continuity had a flat mucosa, but the relative height of the villi was unchanged.* According to STAUFFER (1975) exclusion from intestinal continuity per se did not lead to mucosal changes in minipigs. It is possible that the combination of extrinsic denervation and exclusion from intestinal continuity is needed to bring about a reduction in mucosal thickness.

Endocrine cells

The effect of autotransplantation on the different endocrine cells in the gut has, to the best of my knowledge, not been investigated previously.

Cells containing the peptide hormones enkephalin, CCK (cholecystokinin), somatostatin, neurotensin, GIP (gastric inhibitory peptide) secretin or motilin were counted. Each of the different cell types has its own characteristic distribution pattern. Thus, enkephalin, somatostatin and motilin cells predominated in the crypts, CCK, GIP and neurotensin cells were evenly distributed in the mucosa, while the secretin cells were found almost exclusively on the villi. The transplants were obtained from the proximal jejunum where secretin and motilin cells are less frequent than in the duodenum. The CCK

and GIP cells are fairly numerous in the upper small intestine and are progressively reduced in number distally in the intestine. The somatostatin cells are uniformly distributed all along the small intestine, while the neurotensin cells are few in the duodenum and more numerous in the jejunum and ileum.

The distribution or the frequency of occurrence of the endocrine cells was not influenced by the denervation per se. On the other hand, *the number of endocrine cells decreased significantly in the transplants that were excluded from intestinal continuity,* which is the case regarding autotransplants used for oesophageal substitution.

The tissue investigations have given valuable basic knowledge for the future development of autotransplantation methods, as well as of intestinal homotransplantation, which is going to be an important method of treatment as soon as the immunological problems are solved.

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THE MICROVASCULAR ANASTOMOSIS TO AORTA

An experimental study in the piglet

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The aorta could be an important vessel within transplantation surgery, as it is easily accessible both thoracically and abdominally. Reliable techniques have been established for anastomoses between macrovessels and aorta; e.g. in coronary by-pass surgery and renal artery surgery. Use of a patch technique, whenever possible, makes the anastomoses easier to perform.

Problems arise when a patch cannot be obtained, and a tiny transplant artery is to be microanastomosed to aorta. The problems are mainly secondary to the large difference in wall thickness between the vessels. The thickness of the aortic wall is approximately the same as the outer diameter of the transplant artery. This makes it difficult to achieve an exact adaptation of the intimal layers, which is of the utmost importance for the functional result of the anastomosis. Great importance must also be attached to obtaining the greatest possible circumference to avoid stenosis. Furthermore, good haemodynamic conditions are essential to avoid thrombosis.

This report describes a method for microanastomoses of tiny arteries to the aorta, which to the best of my knowledge has not been described before. The technique was primarily designed for intestinal autotransplantation to overbridge long gap oesophageal atresia.

MATERIAL AND METHODS

The operations were performed in piglets weighing 5 - 7 kg. Endotracheal anaesthesia was used with 50% O_2 and 50% N_2O. In addition Ketamine hydrochloride, Droperidole, Pethidine and Alcuronium chloride were used. The surgical procedures were performed under sterile conditions with instruments designed for pediatric surgery. Microsurgical instruments and an operative microscope (Zeiss OPMI 1) or glasses with 2.5 times magnification were used for the vascular anastomoses.

A total of 10 animals were operated, 4 cases with intrathoracic autotransplantation of a jejunal segment as an oesophageal substitute, and 6 cases with intraabdominal autotransplantation.

Preparation of the autotransplant

The proximal part of the jejunum was exposed through a midline incision. A suitable branch of the superior mesenteric artery with a diameter of approximately 1.5 mm and a corresponding vein of 2 - 3 mm diameter was identified and a wedge resection of about 10 cm of jejunum was performed from these vessels. Neighbouring segments of jejunum became ischaemic and required secondary resection. Bowel continuity was restored with an end-to-end anastomosis in two layers with 6/0 Dexon^{R}. The total length of resected bowel was between 75 and 100 cm.

The artery and the vein were now divided as proximally as possible before removal of the transplant. A siliconized rubber catheter, 1 mm in diameter, was inserted into the artery and perfusion with refrigerated heparinized Perfadex^{R} solution (5 IU Heparin per ml) was started immediately (Fig. 1).

Intrathoracic transplantation

Thoracotomy was performed in the fifth intercostal space on the left side. After opening of the parietal pleura, the aorta, hemiazygos vein and oesophagus were dissected free. The venous anastomosis was performed first. Depending on the caliber of the hemiazygos vein, the anastomosis was made either end-to-end or end-to-side. A continuous over-and-over suture with 9/0 Ethilon^{R} was used for the anastomosis. An anastomosis between the transplant artery and aorta was performed using the method described below. Several cm of the thoracic oesophagus were resected, and the autotransplant was interposed between the oesophageal ends with end-to-end anastomoses sewn in one layer with interrupted silk sutures. The ischaemia time varied from 90 to 120 minutes.

Intraabdominal transplantation

The abdominal aorta and the inferior vena cava were exposed several centimeters cranially from their bifurcations through a long midline incision. An intestinal transplant was created as described above, and perfusion was immediately begun. An end-to-side anastomosis between the transplant vein and the caval vein was sewn with 9/0 continuous Ethilon^{R}. After this, the transplant artery was anasto-

Fig. 1. Jejunal autotransplant during perfusion via 1 mm catheter in the artery.

mosed to the aorta, using the method described below. The abdominal aortic wall was somewhat thinner than the thoracic aortic wall. After the circulation had been restored, the proximal end of the transplant was closed and the distal end was drawn through and sutured to the abdominal wall as a jejunostomy. The time of ischaemia was between 80 and 130 minutes.

The micro artery - aortic anastomosis

After the aorta had been partially clamped with a U-shaped Potts vascular clamp, a suitable opening in the aortic wall was made. A specially constructed pair of vascular scissors (STILLES, Sweden) was used for this purpose (Fig. 2 A and B). With this instrument, it is possible to obtain an oval and coniform opening with a sharp intimal edge in the bottom of the hole (Fig. 3). The blades of the scissors are spiral-shaped. Therefore, the degree of conicity can be varied by holding the scissors at different angles. The size of the opening is determined by the depth of the cut. Efforts were made to achieve an opening of 2 - 2.5 mm diameter.

The mesenterial artery was cut obliquely, and the adventitial layer was removed from the distal mm. The anastomosis between the mesenterial artery and the aorta was sewn continuously over-and-over with 8/0 Ethilon^{R}. The stitches in the aortic wall were rather coarse, but in the mesenterial artery they were very fine. In some instances, one or two extra stitches were needed to seal the anastomosis. After finishing the anastomosis, the transplant artery pointed approximately 60 degrees caudally.

Methods of evaluation

Autopsy was performed on all animals that died postoperatively. The surviving piglets were followed 6 - 8 weeks before they were sacrificed. The transplant was dissected free, along with a few cm of the aorta and corresponding vein, and removed as a specimen.

Angiography was performed with a liquid barium contrast medium (MICROPAQUE^{R}). The contrast was allowed to flow into the transplant under 13 kPa pressure via a catheter inserted into the aorta.

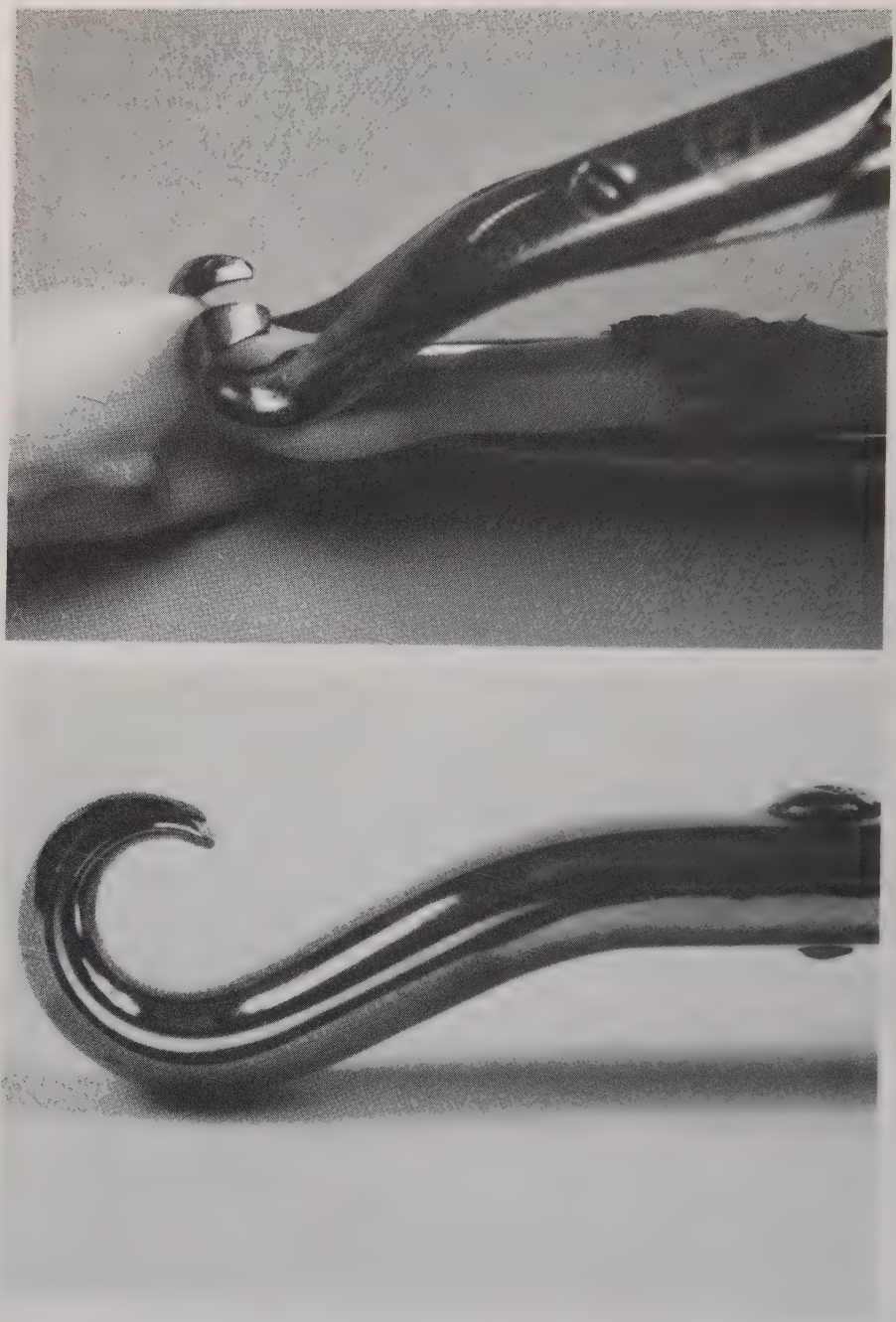

Fig. 2 A. and 2B. Specially designed vascular scissors. Observe the spiral-shape.

Fig. 3. Opening in the aortic wall with sharp intimal edge in bottom performed with a special vascular scissors.

Calibration of the anastomoses was performed before they were opened for inspection.

Macrophotographs of the opened anastomoses were taken.

Occasionally, specimens from the anastomotic area were taken for histology. These were fixed in formaldehyde, dehydrated, embedded in paraffin and stained with haematoxylin-eosin.

RESULTS

All 4 piglets operated intrathoracically died within 8 days. In the first case the autopsy revealed a bluish transplant. No thrombosis or other specific reason for obstruction could be found. The second piglet was found to have a thrombosis in the arterial anastomosis. The third died peroperatively in malignant hyperthermia. The anastomosis had been performed without problems. The fourth piglet died peroperatively because of accidental haemorrhage.

The six animals in the intraabdominal series were followed between three and eight weeks. One piglet died three weeks postoperatively of some infective disease. The transplant was vital and the microvascular anastomoses were patent. In the five sacrificed animals, the transplants were vital.

Calibration of the anastomoses revealed a slight narrowing of the first mm of the transplant arteries, corresponding to the part of the transplant arteries which were situated within the aortic wall.

Angiography confirmed this finding in three cases (Fig. 4).

Macrophotographs of the opened anastomoses showed smooth intimal surfaces and absence of thrombosis in all cases (Fig. 5).

The histological examination demonstrated that the aortic wall adjacent to the anastomosis healed with apparently normal tissue; i.e. smooth muscle, elastic fibers and collagen in normal proportions (Fig. 6).

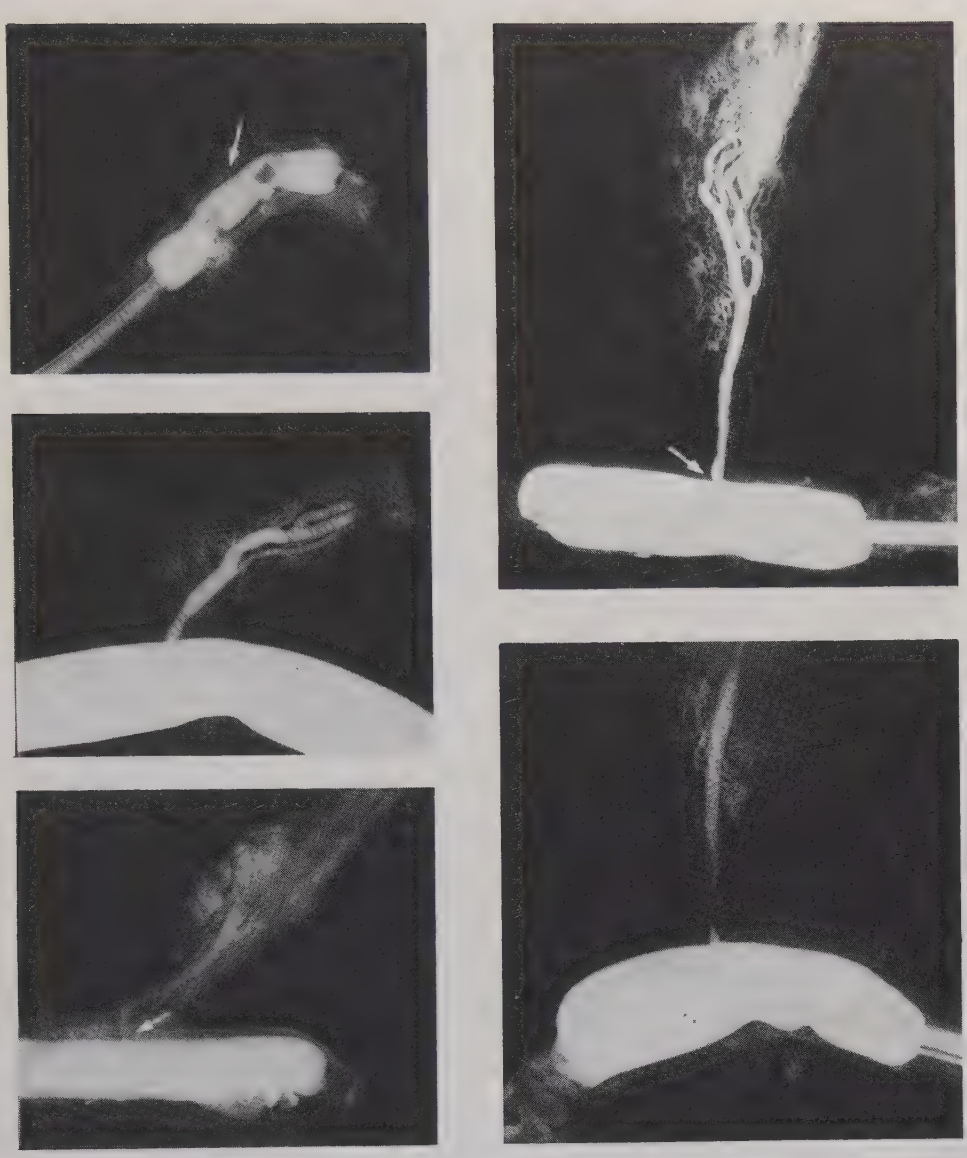

Fig. 4. Angiographies via catheter in the aortic stump. Arrows indicate slight narrowing of first mm of transplant artery.

Fig. 5. Internal view of opened microvascular anastomosis. Note the smooth intimal surface in the anastomotic line.

Fig. 6. Microscopic view showing intimal layer of the transplant artery at top (arrow). Transition to the aortic wall unnoticable.

DISCUSSION

A prerequisite to obtaining patent microvascular anastomoses is a perfect adaptation between the intimal edges. This may be difficult to achieve. In a pilot study, conventional instruments such as a scalpel or scissors were used to make the opening in the aortic wall. It was very difficult to obtain good intimal adaptation under those circumstances, and the results were unacceptable. In order to achieve a sharp and even hole in the aortic wall, special vascular scissors were constructed. They are a smaller version of the instrument described by BERGENTZ et al (1) for surgery of renal artery stenosis. With this instrument a suitable opening can easily be made. It is an advantage that the hole is conical, since the intimal layer in the bottom is well exposed and easy to reach. It would have been preferable to make small even stitches in the aortic wall as well as in the transplant artery, but it was found that the suture often cut through under such circumstances. Coarse, uneven stitches increase the risk of inadequate intimal adaptation, and utmost care must be taken.

Interrupted sutures are widely used in microsurgery, with the argument that they reduce the risk of stricture (3). However, it has been shown that one can use a continuous suture for anastomosing 1 mm vessels without any disadvantages and with a high patency rate (4). One advantage with a continuous suture was the possibility to suture loosely and tighten afterwards. In addition, a continuous suture is faster to perform, which lowers the perfusion time. On the other hand, when the anastomosis is performed in a fast growing individual, interrupted sutures are recommended (6). From this study, it can not be stated which suture method is preferable.

The piglet is a good experimental animal in many respects, especially when the influence of fast growth is to be studied (5). The piglet is, however, not the ideal experimental animal for microvascular surgery. It has a rather large tendency for bleeding and thrombosis (6) and the aortic wall is remarkably fragile. There is reason to believe that the method described should be even more reliable in human practice.

Only one of the 8 piglets, that survived the operation, had an arterial thrombosis. Poor intimal contact was the probable cause in this case, as there were considerable technical difficulties due to movements of the heart, the lungs and the aorta in the thoracic operations. In another piglet, the circulation was insufficient, but no thrombosis or other evident cause could be found. This might be a case of venous microthrombosis, as described by BUSSMAN et al (2) in ileal autotransplants in dogs. It is possible that postoperative administration of heparin might have been of value, but on the other hand, this increases markedly the risk for haemorrhage.

In the remaining 6 piglets, there were no signs of stricture or thrombosis. Calibration of the anastomoses showed narrowing of the first mm of the transplant arteries. This could be documented angiographically in only 3 of the cases. However, angiographic examinations are difficult to interpret accurately in all cases, as the projections have to be strictly tangential.

During the operation, a rather large coniform hole was made in the aorta and the anastomosis was sewn in the bottom of it. During healing, the tissue defect in the aortic wall was replaced by normal tissue. It seems probable that the luminary width adapted to the perfusion demand of the transplant, since the artery of the transplant is heavily overdimensioned for the diminutive capillary bed of the transplant. This hypothesis is supported by the fact that the vascular supply was sufficient during a long follow-up time in spite of the virtual narrowing. If this hypothesis is true, there should be no narrowing if one transposes the mesenterial artery to the aorta without any intestinal resection. This may be a topic for further study.

This investigation has proven the possibility of anastomosing 1.5 mm arteries end-to-side to the aorta with an acceptable frequency of patent anastomoses and without strictures. The method ought to be useful in the treatment of long gap oesophageal atresia with intestinal autotransplantation. There ought to be space for several other applications both thoracically and abdominally. The end-to-side microvascular anastomosis to the aorta is much more difficult to perform than microvascular anastomoses between equally sized vessels, and

therefore makes great demands on the surgeon who must be welltrained in microsurgery. The specially designed scissors may almost be considered a prerequisite for good results.

ABSTRACT

A method to perform microanastomoses between 1.5 mm arteries and the aorta is described.

The technical problems can be related to the big difference in wall thickness between the aorta and the microartery and to difficulties to cut a suitable opening in the aortic wall. A specially constructed instrument, vascular scissors with spiral-shaped blades, was used. The scissors cut an oval coniform opening with sharp edges in the aortic wall, which made it possible to gain close intimal contact.

Using the method, intraabdominal autotransplantation of a jejunal segment was performed in 6 piglets. Intrathoracic autotransplantation of a jejunal segment as an oesophageal substitute was performed in 4 piglets. Eight piglets survived the operation, six were followed between 3 and 8 weeks. Thrombosis of the microarterial anastomosis occurred in 1 case.

The results have proven the possibility of anastomosing 1.5 mm arteries end-to-side to the aorta with an acceptable frequency of patent anastomoses and without strictures.

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RECONSTRUCTION OF THE THORACIC OESOPHAGUS USING AUTOTRANSPLANTED SMALL INTESTINE

An experimental study in the piglet

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Substitution of cervical oesophageal defects with revascularized intestinal autotransplants has been performed in man since 1959 (SEIDENBERG et al 1959). During the sixties occasional cases were reported, but recently large materials with good results have been reported (UEMICHI et al 1977, GERMAIN et al 1980). Similar methods to overbridge thoracic oesophageal defects have been tried experimentally with the purpose to treat oesophageal malignancy (MEIER 1968, GÖBER et al 1972, KOZUSCHEK et al 1972, DRAGOJEVIC et al 1975, ODHIAMBO 1979). In most cases the transplant vessels were anastomosed to internal thoracic or intercostal vessels. With one exception (MEEUWIS et al 1980), these reported series have been afflicted with a high mortality and morbidity. One of the remaining significant problems is to avoid kinking of the proportionately long vascular pedicle.

The aim of the present study was to investigate the technical problems concerned with intestinal autotransplantation in small individuals, using anastomoses directly to the aorta and azygos or caval vein respectively. The procedure is adapted for minute vessels. In addition it shortens the vascular pedicle and lessens the risk of kinking. The technique of microanastomosing tiny arteries to the aorta was studied and thoroughly described elsewhere (MALMFORS 1981). This study was performed with rapidly growing animals since one of the clinical applications is to overbridge long-gap oesophageal atresia in newborns.

MATERIAL AND METHODS

Thirtytwo piglets of ordinary Swedish landbreed were used. At operation they were between 3 and 4 weeks of age and weighed 4.7 - 8.4 kg (mean 6.5 kg). They were all operated with the same method, but minor divergencies were necessary. Anaesthesia was induced with Ketamine hydrochloride and Droperidole i.m. Mechanical ventilation with 50% O_2 and 50% N_2O was used, and Alcuronium chloride for muscular relaxation. Twentyone of the 32 operations were performed by one surgeon with an assistant. At eleven of the operations two surgeons took part. The operations were performed under sterile conditions with pediatric surgical and microsurgical instruments.

Interposition of an intestinal autotransplant was performed after partial resection of the thoracic oesophagus. The operative time ranged from 5 to 7 hours. During the operation 5 million IE depot penicillin and 0.5 g Cephalotine were given. Cephalotine was continued for three or four days postoperatively.

I.v. perfusion with dextran 70 6% 50 ml/kg b.w. and 500 ml Ringer glucose solution was started during the operation. For another three or four days 100 ml Ringer glucose/kg b.w. was administered i.v. The enteral feeding varied. In the first third of the series the piglets had a transanastomotic gastric tube. The piglets in the second third had gastrostomies and the last third were allowed to drink freely.

The animals that died during the operation were not examined further; whereas, an autopsy was performed on those animals that died postoperatively. The survivors were examined with barium swallow X-ray and selective angiography. The barium swallow was performed under general anaesthesia. The contrast was administered through a catheter in the proximal oesophagus. The swallow was studied under fluoroscopy and documented. The angiographies were performed via a catheter in the femoral artery. An aortography was carried out first, after which the transplant artery was selectively catheterized if possible.

Operative technique

A suitable segment of the small intestine was exposed through a midline laparotomy. The ideal was to find an artery approx. 1.5 mm and a corresponding vein 2 - 3 mm in width. The transplants were taken from the upper half of jejunum in 30 cases and from ileum in 2 cases. A wedge-shaped resection of mesentery and approx. 10 cm of intestine was performed. Adjacent segments of the intestine became ischaemic, thereby requiring additional resection. The total resected length ranged from 0.5 to 1 m. Bowel continuity was restored with an end-to-end two-layer suture with Dexon^{R} 6/0. The vascular pedicle was divided as proximal as possible. A 1 mm in diameter siliconized rubber catheter was inserted in the artery (Fig. 1) and perfusion with refrigerated dextran - electrolyte solution with heparin 5 IE per ml was started immediately.

Fig. 1. Autotransplant during perfusion via 1 mm catheter in the artery.

Thoracotomy in the left fifth intercostal space was performed. The hemiazygos vein, the first part of the descending aorta and oesophagus were dissected free. The venous anastomosis was performed between the transplant vein and the hemiazygos vein. In most cases an end-to-end suture was possible, but in some cases an end-to-side anastomosis was preferred, because of a big difference in width between the two vessels. Microsurgical instruments and operative microscope (Zeiss OPMI 1) or magnification glasses (2.5 x) were used for construction of the anastomoses with continuous over-and-over sutures of silk or Ethilon^{R} 8/0 - 10/0.

The microanastomoses between the transplant artery and aorta was performed end-to-side. The aorta was partially clamped with a Potts U-shaped vascular clamp. An oval opening in the aortic wall was made with scissors or a scalpel. It was difficult to obtain sharp edges, as the aortic wall was thick but fragile, showing a tendency to rift. Specially constructed scissors with spiral-shaped blades that create an oval opening with sharp even edges were used in the latest operations (BERGENTZ 1979, MALMFORS 1981). The transplant artery was cut obliquely and anastomosed to the aorta with a continuous 8/0 - 9/0 silk or Ethilon^{R} suture. Pulsating bleeding from the transplant was the criterion of successful revascularization.

Approximately 5 cm of oesophagus was resected and the transplant was trimmed to the appropriate length. The anastomoses between the transplant and oesophagus were sewn in one layer with interrupted sutures of 6/0 silk (Fig. 2). The vascular pedicle was usually longer than the distance between aorta and oesophagus, thereby creating a risk for kinking. However, great effort was made to avoid it. Finally, the thoracic wall and the abdominal wall was closed.

RESULTS

The revascularization was successful in 19 of the 32 cases; i.e. there was pulsating bleeding from the transplant and no signs of venous obstruction were seen. A remarkable oedema of the bowel wall with transexudation into the intestinal lumen was seen in several transplants.

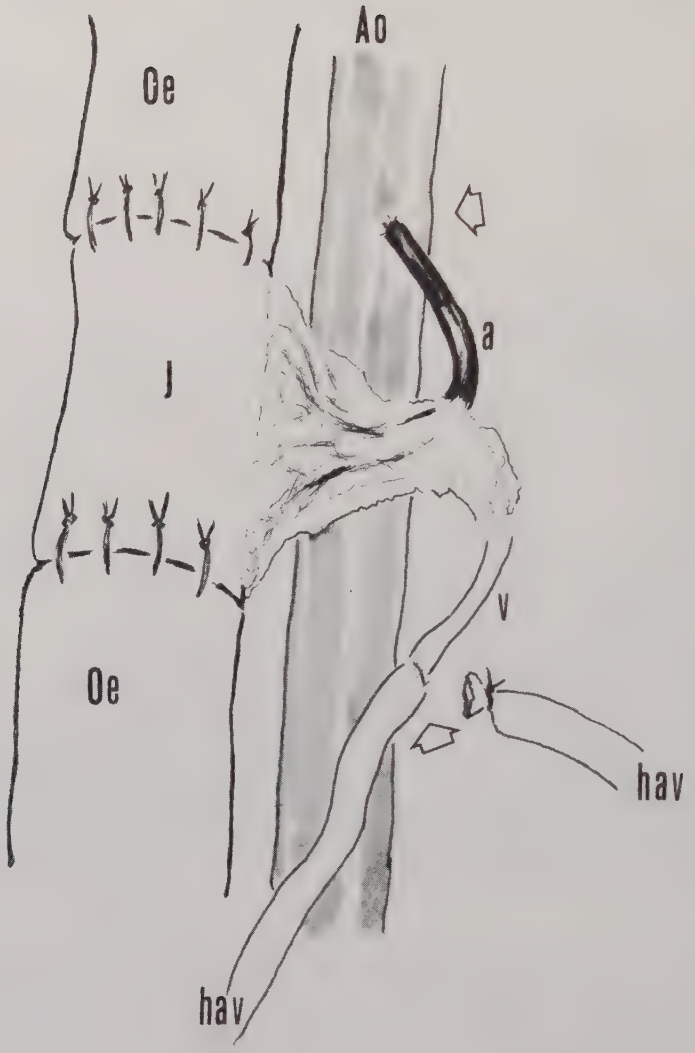

Fig. 2. Schematic representation of operative procedure.
Oe = oesophagus, J = jejunal autotransplant, a = transplant artery, Ao = aorta, v = transplant vein, hav = hemiazygos vein.

The series was charged with a high mortality. Four animals survived more than two weeks. These cases originate from the last part of the series and will be described below.

Different complications to the anaesthesia killed 10 piglets. The operative procedure was finished before they died in most cases. A few of them died with acidosis. Bleeding was the probable cause of death in 3 cases. One piglet died in postoperative intestinal obstruction.

The microanastomosis to the aorta failed in 8 cases resulting in sacrifice of the piglets. This was the case in 5 of the 8 first operations. Transplant necrosis during the first postoperative week was the cause of death in 6 cases. Three of these had a thrombosis in the arterial anastomosis. In one case there was a venous thrombosis, and in 2 cases the transplant was necrotic, but no thrombosis could be found macroscopically.

All four of the piglets described below survived more than two weeks and had jejunal transplants. Their weight varied between 6.2 and 6.9 kg. The perfusion time was 2 hours ± 10 minutes.

Case I. This piglet had diarrhoea postoperatively and did not gain weight. Barium swallow two weeks postoperatively showed normal passage through the transplant, but a minor leak in the proximal anastomosis. Selective angiography showed adequate transplant perfusion (Fig. 3). The barium examination was misinterpreted as a fistula, and the pig was sacrificed. The transplant was vital (Fig. 4). The vascular anastomoses were intact, and no thrombosis was observed. The contrast leak was a pouch between two of the sutures with a very slight inflammatory reaction. This piglet would have had a good chance for longterm survival.

Case II. Ordinary postoperative course. The barium swallow after three weeks showed good passage and no signs of leakage. The transplant artery was not seen at the aortography. An attempt to selective catheterization failed. The animal died 5 weeks postoperatively in a major hematemesis. At autopsy, a connection was found between the

Fig. 3. Angiographic view of jejunal autotransplant interposed as oesophageal substitute. Catheter in aorta.

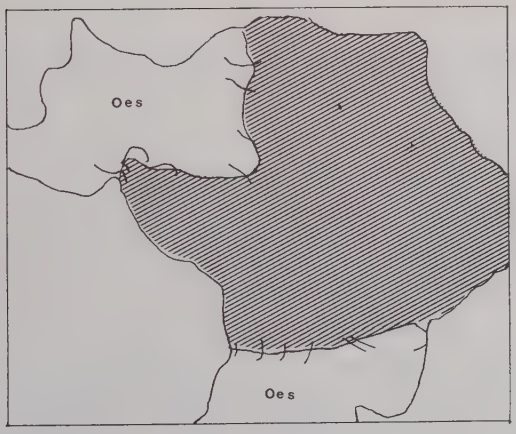

Fig. 4 A. Vital jejunal autotransplant at sacrifice two weeks post-operatively in case 1.

B. Autotransplant represented by shadowed area. Oes = oesophagus.

aortic anastomosis and one of the anastomoses between the transplant and oesophagus. The transplant was vital. The area was strongly inflammatoric, especially around the silk sutures. The microanastomoses were not identifiable.

Case III. The first two weeks elapsed uncomplicated. Barium swallow and aortography after 3 weeks showed a leak in the proximal anastomosis and no arterial perfusion of the transplant. A necrotic transplant was found at sacrifice. The microanastomoses were not possible to identify.

Case IV. The first two postoperative weeks were uneventful. A barium swallow at this time showed stenosis of the distal anastomosis and an irregular transplant wall. No arterial circulation could be demonstrated at angiography. The transplant was necrotic at autopsy. The vascular anastomoses could not be identified in this case either.

DISCUSSION

The method described has some theoretical advantages in the treatment of long gap oesophageal atresia. The main one is the saving of intestinal tissue. The use of ileocolic vessels in man means loss of no more intestine than is needed for the transplant. All interposition methods now in clinical use require transfer of a huge amount of tissue from the abdomen into the thoracic cage. In WATERSTONS (1957) and SHERMANS (1957) bowel interposition methods, almost half the colon is used to overbridge 5 - 6 cm oesophageal defect. Contrary to most other methods, all oesophageal tissue is maintained and used for reconstruction, and the function of the cardia is left intact. Contrary to nonvascularized autotransplants (HALSBAND et al 1977, OESCH et al 1980) the oesophageal defect is corrected with a vital organ with elasticity and peristalsis. The main disadvantage is the risk of necrosis, which was very high in this series, but should be considerably lower in clinical use for reasons discussed below. The risk of necrosis is probably comparable to that of the interposition methods. Autotransplantation also implies better possibilities for reoperation than the colon interposition methods, where reoperation means an almost total loss of the colon.

The growth of the individual is an important factor in the evaluation of a pediatric surgical operative procedure. The piglet is a suitable experimental animal because of its rapid growth, which reproduces the entire human growth period in half a year (KORNFÄLT 1973, NELSON 1976). On the other hand, previous experience with cervical autotransplantation in the piglet was not encouraging (OKMIAN 1976). The porcine vessels are rather fragile, which is a disadvantage in microvascular surgery. The dog is probably a more suitable experimental animal. The experience gained from this series supports this view.

The anaesthesia used was applied to several hundred piglets in earlier experimental series with good results (KORNFÄLT 1973, NELSON 1976, KULLENDORFF 1980). However, the operations in this report have meant simultaneous thoracotomy and laparotomy, and the operative time has been considerably longer. The deaths caused by acidosis were due to hypothermia. After the introduction of heat pads, no more deaths occurred of this reason.

Vascular surgery always means a risk of bleeding, even if every effort is made to avoid it. The three deaths caused by peroperative haemorrhage could have been avoided if blood transfusions had been available.

Improper microsurgical technique was the cause of some failures, especially in the beginning of the series. To microanastomose microarteries to the aorta in the depth of the thoracic cage of a piglet is a difficult task, quite different from microvascular anastomoses in mice or guineapigs. The benefit of an operative microscope is limited, since the operative area constantly fluctuates out of focus because of movements of the lung, the heart and the aorta. A low magnification or magnification glasses appeared to be more suitable. A certain weariness during the long tedious operations might have contributed to some failures.

The most important single factor in avoiding thrombosis is an exact intimal contact between the vessels. The opening in the aorta must have smooth, even edges. This is difficult to obtain with conventional instruments, but rather easy with the special vascular scissors

used in the latter part of this series. Defective intimal contact has probably contributed to the three cases of arterial thrombosis. The cases of transplant necrosis without macroscopic thrombosis were probably due to venous microthrombotism, as described by BUSSMAN et al (1972) in ileal autotransplants in dogs. The thrombosis prevention given, i.e. dextran i.v. and heparin in the perfusion solution, should perhaps be supplemented with heparin postoperatively. However, this was not given, because of the risk of bleeding. It is worthwhile to question if perfusion and cooling of the transplant was necessary. GERMAIN et al (1980) performed cervical intestinal autotransplantation without any perfusion in 30 dogs with 27 successes.

Even though the survival rate in this study is unsatisfactory, the results support the view that intestinal autotransplantation for oesophageal reconstruction in cases of long gap oesophageal atresia could be developed into a clinical method. The access to the advanced facilities of human medical care would certainly mean a much higher survival rate. The most important factors needed to improve the low success rate found in this study are an anaesthetist, two operative teams, the possibility to give blood transfusion, and adequate post-operative supervision. As the operative procedure is technically demanding, careful preparation and practice is mandatory, if this kind of operation is to be performed with a good safety margin. A possible clinical application in the future should be centralized, as each unit treats too few cases to achieve sufficient experience.

ABSTRACT

A technique to overbridge thoracic oesophageal defects with revascularized intestinal autotransplants was evaluated in 32 piglets. A microvascular end-to-side anastomosis was performed between the 1.5 mm transplant artery and the aorta. Specially constructed vascular scissors were used to cut a suitable opening in the aorta. The venous anastomosis was performed between the transplant vein and the hemiazygos vein. After partial oesophageal resection the revascularized autotransplant was interposed between the oesophageal ends and anastomosed. The series was charged with a high complication rate. The study has, however, proven that technical prerequisites exist to perform this kind of operation.

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Peptide-containing Neurons Intrinsic to the Gut Wall

An Experimental Study in the Pig

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Summary. Nerve fibers containing substance P, VIP, enkephalin or somatostatin are numerous in the porcine gut wall. They are particularly numerous in the submucosal and myenteric plexuses where peptide-containing cell bodies are also observed. Peptide-containing nerve fibers occur also in the vagus nerves, suggesting that the gut receives an extrinsic supply of peptidergic nerves. The extrinsic contribution to the peptide-containing nerve supply of the gut wall has not yet been quantitatively assessed. In an attempt to clarify this question pigs were subjected to bilateral subdiaphragmatic vagotomy. Another group of animals was subjected to complete extrinsic denervation by autotransplantation of a jejunal segment. The pigs were killed at various time intervals after the operations; the longest time interval studied was four months. Following vagotomy the innervation pattern of the jejunum appeared completely unaffected. Following complete extrinsic denervation the adrenergic nerve fibers disappeared, while peptide-containing and acetylcholinesterase-positive nerve fibers remained apparently unaltered. This was confirmed chemically in the case of substance P.

The motor activity of smooth muscle from the jejunum was studied in vitro. At low stimulation frequencies the smooth muscle from control jejunum responded by relaxation; upon cessation of stimulation a contraction occurred. With increasing stimulation frequencies the duration of the relaxation decreased; at high frequency stimulation only a contraction was recorded. In the autotransplant low frequency stimulation induced no or only a weak relaxation; high frequency stimulation induced contraction. After cholinergic and adrenergic blockade, the muscle responded with relaxation at all frequencies; the response was similar in innervated and denervated specimens. On the whole, the effects of extrinsic denervation on the motor activity of smooth muscle from porcine jejunum were minor, possibly reflecting the high degree of autonomy of the gut.

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Nerve fibers in the gut have their origin either in ganglia located outside (extrinsic nerves) or within the gut wall (intrinsic nerves). Apart from classical adrenergic and cholinergic nerve fibers, the gut harbours a variety of peptide-containing fibers (cf. Sundler et al. 1980). Among recognized peptide-containing nerve fibers are those storing substance P, vasoactive intestinal polypeptide (VIP), enkephalin or somatostatin (cf. Furness and Costa 1980). The fact that peptide-containing nerve cell bodies occur in the intramural ganglia (Larsson et al. 1976; Costa et al. 1977; Sundler et al. 1977; Alumets et al. 1978) suggests that the nerve fibers are intrinsic to the gut. Experimental evidence also supports this view (Franco et al. 1979; Schultzberg et al. 1978; Jessen et al. 1980). However, additional peptide-containing nerve fibers may originate from sources extrinsic to the gut, and recent reports indicate that the vagal trunks possess such fibers (Lundberg et al. 1978, 1979).

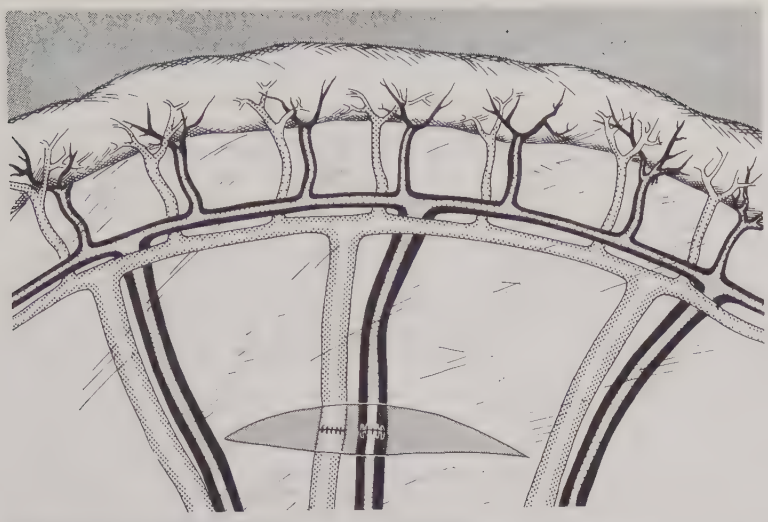
Materials and Methods

Surgical Procedures

I. Autotransplantation of Jejunal Segments. In one series of experiments nine piglets weighing approximately 6 kg were operated according to a modification of a method originally designed for autotransplantation of jejunum as substitute for the thoracic oesophagus. The piglets were anesthetized with N_2O, pethidine, droperidol (Dridol®), ketamine (Ketalar®) and alcurone (Alloferin®). At laparotomy the proximal half of the jejunum was exposed. A mesenterial artery of approximately 1.5 mm width and an adjoining vein, 2–3 mm in diameter, were isolated. These vessels supplied 80–90 cm of jejunum. The central 15 cm were freed together with the vascular pedicle. The anoxic parts of jejunum were resected and the vital ends were joined by an end-to-end anastomosis. The vascular pedicle was divided and the transplant was perfused via the mesenterial artery with a refrigerated solution of dextran, electrolytes (Perfadex®, Pharmacia, Uppsala, Sweden) and heparin (5 IU ml) by means of a siliconized rubber catheter. The vein was anastomosed end-to-side to the caval vein a few cm cranial to its bifurcation. The anastomosis was sutured with 9/0 ethilon. The mesenterial artery was cut obliquely and a suitable hole was made in the aortic wall. An end-to-side anastomosis was then performed with a 8/0 ethilon suture. The vascular clamps were removed, and the anastomoses were inspected for leakage. The proximal end of the transplant was closed, and the distal end was sutured to the abdominal wall as a jejunostomy. The time from the beginning of the perfusion until circulation was reestablished varied from 60 to 90 min.

In order to eliminate possible effects of ischaemia another method of autotransplantation was developed. Thus, six piglets were operated in the following manner: At first a suitable mesenterial artery of 1.5 mm width and its adjoining vein, 2 to 3 mm in diameter, were dissected free from surrounding tissue. The next step was to divide the vein between clamps and immediately resuture it with continuous 9/0 ethilon sutures. Thereafter the same procedure was performed with the artery (Fig. 1a). Microvascular equipment and technique were used. During the time needed for the vascular anastomoses the transplant was sufficiently supplied via the arcade collaterals. A wedge-shaped resection of the mesenterium was then performed, and the gut was divided at two sites situated 10 to 20 cm from each other (Fig. 1b). Thus a free autotransplant, which had never been ischaemic, was created. The autotransplants were either resutured into the bowel continuity (Fig. 1c) or applied to the abdominal wall as jejunostomies.

Fig. 1a–c. Autotransplantation of a jejunal segment without producing ischaemia. **a** Vessels are divided and resutured. Sufficient collateral circulation. **b** Wedge shaped resection creates an autotransplant that is already revascularized. **c** Bowel continuity is restored



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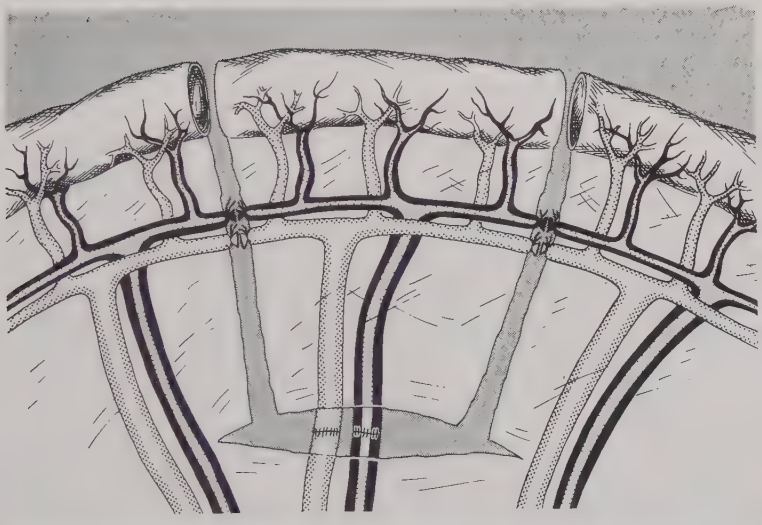

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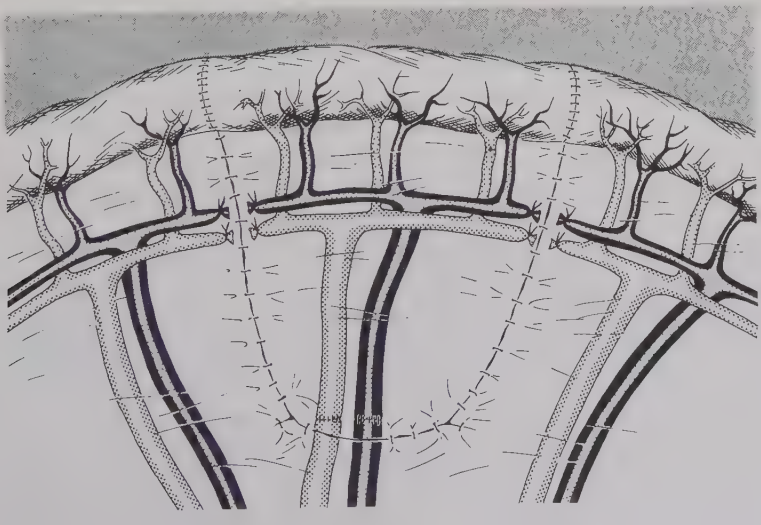

c

The piglets were sacrified 1, 2 or 4 months after operation. The autotransplant with its vascular pedicle was dissected free while the animal was under anesthesia. Tissue specimens were taken from the autotransplant and from the jejunum in situ close to the anastomosis. For histochemical analysis specimens were frozen in a mixture of propane and propylene cooled to the temperature of liquid nitrogen. Alternatively, specimens were immersed overnight in 4% formaldehyde in 0.1 M phosphate buffer, pH 7.2, rinsed in buffer containing 5% sucrose, and frozen as above for cryostat sectioning. Specimens for chemical analysis were immediately frozen in liquid nitrogen. Specimens for in vitro studies of motor activity were placed in +4°C Kreb's solution (for composition, see below).

II. Division or Ligation of the Vagal Trunks. In another series of experiments four piglets were vagotomized to evaluate the vagal contribution of peptidergic nerves to the gut. Vagotomy was performed abdominally. The two trunks were ligated or divided. In the piglet that was planned to survive for the longest time, the vagotomy was combined with a pyloroplasty. The piglets were killed after one day, one week, three weeks and six weeks, respectively. Specimens were taken from the vagus (both sides of the ligature) and from the jejunum.

Histochemical Procedures

Specimens were freeze-dried and fixed by exposure either to gaseous formaldehyde for 1 h at 80°C (Björklund et al. 1972) or to gaseous diethylpyrocarbonate (DEPC) for 3 h at 55°C (Pearse et al. 1974). Deparaffinized sections (6 µm thick) from the DEPC-fixed material and cryostat sections (20 µm thick) from the formalin-fixed material were processed for the immunohistochemical demonstration of substance P, VIP, enkephalin or somatostatin. For details of the antisera used, see Table 1. The indirect immunofluorescence method of Coons et al. (1955) or the peroxidase-antiperoxidase (PAP) technique of Sternberger (1974) were used. Sections incubated with antiserum inactivated by addition of the respective antigen in excess (10–100 µg of antigen per ml diluted antiserum) served as controls. Immunofluorescence was observed in a fluorescence microscope equipped with epi-illumination system and with filters selected to give peak excitation at 490 nm. For the demonstration of adrenergic nerve fibers sections were cut from formaldehyde-treated specimens. These sections were mounted in Entellan and examined in a fluorescence microscope for formaldehyde-induced fluorescence using filters giving peak excitation at 410 nm (Björklund et al. 1972).

For the demonstration of acetylcholinesterase-positive nerve fibers cryostat sections from fresh-frozen tissue specimens were kept in a Na_2SO_4 solution (24%) at 37°C overnight. They were then incubated for 5 h at room temperature in acetylthiocholine iodide (Fluka AG, Buchs AG, Switzerland) for acetylcholinesterase staining according to the method of Koelle as modified by Holmstedt (1957).

Table 1. Characteristics of antisera used

Antisera raised against	Code no.	Working dilution		Source	References
		Immuno-fluores-cence	PAP staining		
Leu-Enkephalin	170	1 : 60	1 : 240	K.-J. Chang, Burroughs Wellcome Research Triangle Park, N.C.	Alumets et al. 1978 Miller et al. 1978
Somatostatin	19578	1 : 80	1 : 1,280	M.P. Dubois, Institut Nationale de la Recherche Agronomique, Nouzilly, France	Dubois 1975; Alumets et al. 1977
Substance P	JK1	1 : 320	1 : 2,560	P. Emson, Cambridge University, Cambridge, England	
VIP	5603	1 : 80	1 : 5,120	J. Fahrenkrug, Bispebjerg Hospital, Copenhagen, Denmark	Fahrenkrug and Schaffalitzky de Muckadell 1977; 1978

The specificity of the staining was confirmed by using Mipafox (N,N-diisopropyl-phosphorodiamid fluoride, 4×10^{-6} M) (FOA, Stockholm, Sweden) to inhibit butyrylcholinesterase, and 284 C51 (1,5 bis allyldimethyl-ammonium-phenyl) pentan-3-one dibromide, 10^{-5} M) (Wellcome Res. Labs, Beckenham, England) to inhibit acetylcholinesterase. The sections were counterstained with eosin and mounted in Permount.

Procedure for Studying Isotonic Mechanical Activity

Tissue specimens were kept in Krebs' solution at 4° C for 1 to 5 h. Mucosa and submucosa were removed and strips of muscle measuring approximately 20 × 3 mm were cut parallel to the longitudinal layer of the jejunum. The strips were mounted vertically on a holder in a 25 ml organ bath thermostated at 37° C. One end was attached to a rigid support and the free end of the muscle was attached to a lever connected via a spring to a Grass FT 03 force displacement transducer for isotonical registration of mechanical activity. The load on the muscle was in all experiments 0.5 g. The muscle was left in the bath to equilibrate for 1 h before experimentation started. Plantinum electrodes were placed on either side of the muscle with a constant electrode distance of 5 mm. The electrodes were connected to a Grass S4C stimulator for field stimulation with square wave pulses (8 V/cm; 1 msec duration). The polarity of the electrodes was shifted after each stimulation period to avoid polarization. The mechanical activity of the preparation was recorded continuously throughout the experiment on a Grass model 7 Polygraph. The bathing solution had the following composition in mM: NaCl 133, $NaHCO_3$ 16.3, KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5, glucose 7,8, and aerated with a mixture of 7% CO_2 in O_2 giving a pH of 7.2–7.3. The experiments were always paired with control muscle and autotransplant muscle in the same bath. Eight muscle pairs from four pigs were tested and preparations that did not show regular spontaneous activity were discarded (4 pairs out of 8). The following drugs were used: atropine sulphate (ACO Inc.), guanethidine sulphate (CIBA-Geigy) and tetrodotoxin (Sankyo Co. Ltd).

Chemical Analysis

Tissue specimens were cut in small (1 mm) pieces in the frozen state and immediately immersed in at least 10 volumes of boiling distilled water acidified with hydrochloric acid to pH 4. After 10 min boiling and subsequent centrifugation of the extracts, the supernatants were decanted and then stored at −20° C until assayed for substance P (Nilsson et al. 1975b). The substance P antiserum (K 25) was used in a final dilution of 1 : 350,000, and ^{125}I-tyr^8-substance P (specific activity 500–600 μCi/μg) served as tracer. The extracts were routinely assayed in duplicates at three different dilutions. The slopes of the dilution curves prepared with tissue extracts were parallel to the substance P – standard curve. Synthetic substance P was obtained from Beckman, Geneva, Switzerland. Appropriate control tubes were incubated for all standard and sample determinations to establish the extent of apparent binding in the absence of antibody; the correction factor varied between 1.9% and 3.7%. The sensitivity of the assay was 2.5 pg (1.85 fmole).

Results

Chemical and Histochemical Observations

In the intact jejunum, nerve fibers displaying VIP, enkephalin, substance P or somatostatin immunoreactivity were numerous in the myenteric plexus (Fig. 2) and occurred scattered in the smooth muscle (Fig. 3). VIP-, substance P- and somatostatin-immunoreactive fibers and nerve cell bodies were regularly observed also in the submucosal plexus. Only VIP-containing fibers were numerous in the mucosa (Fig. 4). In the vagally denervated jejunum and in the autotransplants the distribution and frequency of occurrence of the various types of peptidergic nerve fibers did not seem to differ from that of the intact jejunum, indicating that all four types of nerve fibers originate within the gut (Figs. 2, 3). The innervation pattern of the autotransplants did not change within the time intervals studied, and was the same regardless of which of the two surgical procudures was used, suggesting that

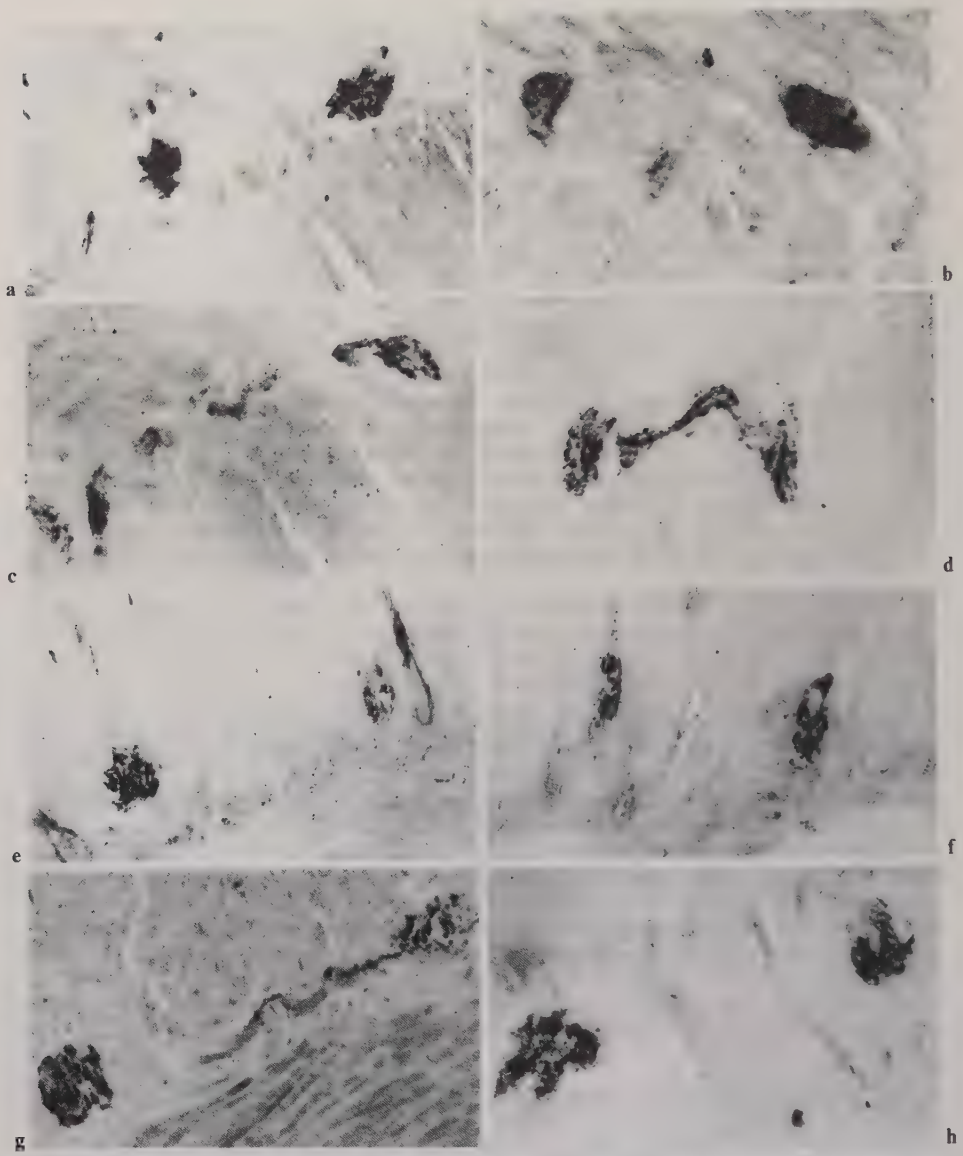

Fig. 2a–h. Immunohistochemical demonstration (PAP technique) of peptide-containing nerve fibers in the myenteric plexus of jejunum in situ (*left panel*) and autotransplant 4 months after operation (*right panel*); **a** and **b** substance P, **c** and **d** VIP, **e** and **f** enkephalin, **g** and **h** somatostatin. There was no conspicuous difference between autotransplant and jejunum in situ with respect to the supply of peptide-containing nerve fibers. ×160

neither transient ischaemia nor loss of bowel continuity affects the supply of peptide-containing nerve fibers. Chemical determinations of substance P revealed slightly elevated levels in four of the five autotransplants (isolated jejunal loops) compared to the innervated jejunum of the same animal: mean concentration

Fig. 3a–f. Sections from porcine jejunum in situ (*left panel*) and from jejunal autotransplant 4 months after operation (*right panel*). **a** and **b** Fluorescence histochemical demonstration of adrenergic nerve fibers. Such fibers are numerous around blood vessels in the submucosa of jejunum in situ. They are absent in the autotransplant. Yellow-fluorescent enterochromaffin cells at the upper margin of the figures. ×225. **c** and **d** Acetylcholinesterase-containing nerve fibers are abundant in the myenteric plexus and smooth muscle of jejunum in situ as well as in autotransplant. ×135. **e** and **f** Immunohistochemical demonstration of substance P-containing nerve fibers (PAP technique) in circular smooth muscle of jejunum in situ and of autotransplant. Extrinsic denervation does not alter the frequency and distribution of substance P-containing fibers. ×225

Table 2. Concentration of immunoreactive substance P (pmol/g) in control jejunum and autotransplanted jejunum of 5 pigs 2 months after operation

Jejunum in situ	Jejunum autotransplant
3.43	4.15
7.85	15.56
6.46	7.97
13.55	7.92
1.98	2.92
Mean ± S.E.M.	
6.65 ± 2.02	7.70 ± 2.20

7.7 pmol/g ± 2.2 (S.E.M., $n = 5$) versus 6.7 ± 2.0 (S.E.M., $n = 5$). This difference is not statistically significant (Table 2).

Adrenergic nerve fibers occurred in all layers of the intact jejunum; they were numerous in the myenteric and submucosal plexuses and around blood vessels. They were few or moderate in number in the smooth muscle and around the intestinal crypts. In the vagally denervated jejunum adrenergic nerve fibers remained visibly unaffected, regardless of the time after denervation. They were completely absent from the autotransplants (Fig. 3), indicating that all adrenergic fibers are derived from ganglia situated outside the gut wall. Acetylcholinesterase-positive nerve fibers were abundant in the jejunal wall. Vagal denervation had no effect on these nerves, and there was no overt difference in the distribution and frequency of such fibers in the autotransplants compared with the intact jejunum (Fig. 3).

In the vagal trunk VIP and substance P nerve fibers were the predominating types of peptide-containing fibers. Upon ligation there was a pronounced accumulation of immunoreactive VIP and substance P in swollen fibres in the vagus trunk on both sides of the ligature, but especially above the ligature (Fig. 5). Somatostatin- and enkephalin-containing fibers were only occasionally detected in the vagus, and there was no conspicuous accumulation of somatostatin or enkephalin immunoreactive material either above or below the ligature.

Spontaneous and Neurally Evoked Activity of the Muscle Strips

When working against the load, strips of muscle from the jejunum showed a regular spontaneous activity. The autotransplants (isolated jejunal loops) displayed a less regular motor activity with a lower frequency and a tendency to lower amplitude. The spontaneous activity was not inhibited by tetrodotoxin, a drug known to block nerve conduction specifically (Kao 1966). Upon electrical field stimulation at 5 Hz the control muscle responded by a relaxation persisting throughout the stimulation period. Upon cessation of stimulation, a contraction occurred. With increasing stimulation frequencies the duration of the relaxation decreased and at high frequency stimulation only a contraction was seen (Fig. 6). In the autotransplants stimulation at 5 Hz induced no or only a weak relaxation, while stimulation at higher frequencies induced contraction. After cholinergic and adrenergic blockade by atropine (10^{-6} M) and guanethidine (5×10^{-6} M) the muscle responded by

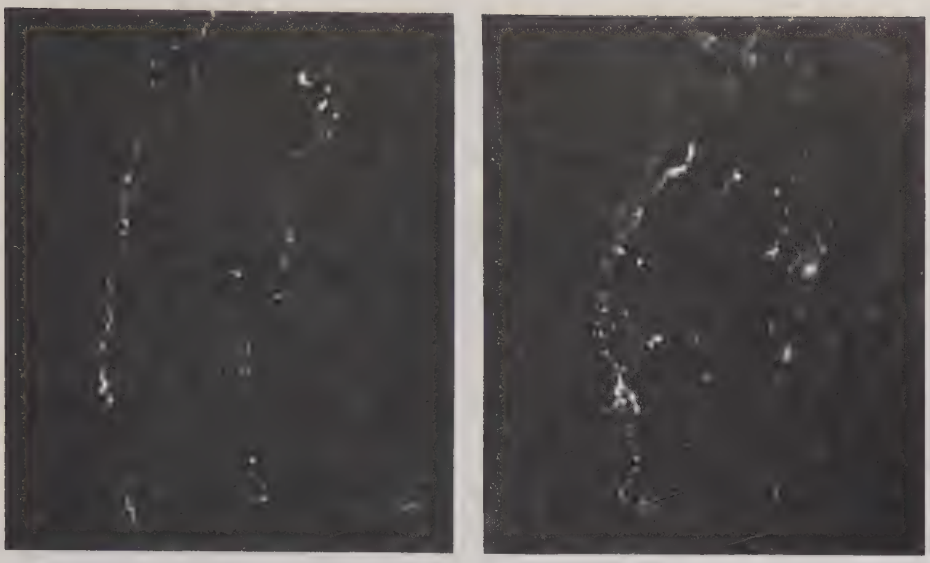

Fig. 4. VIP-containing nerve fibers demonstrated by immunofluorescence in the mucosa (villous core) of jejunum in situ (*left*) and autotransplant 4 months after operation (*right*). There was no apparent loss of VIP nerve fibers following extrinsic denervation. × 200

Fig. 5a–d. Longitudinal sections through the vagus immediately above (*left panel*) and below (*right panel*) a ligature, applied for one week. Numerous swollen nerve fibers displaying intense substance P (**a** and **b**) or VIP (**c** and **d**) immunoreactivity occur both above and below the ligature. PAP technique. × 270

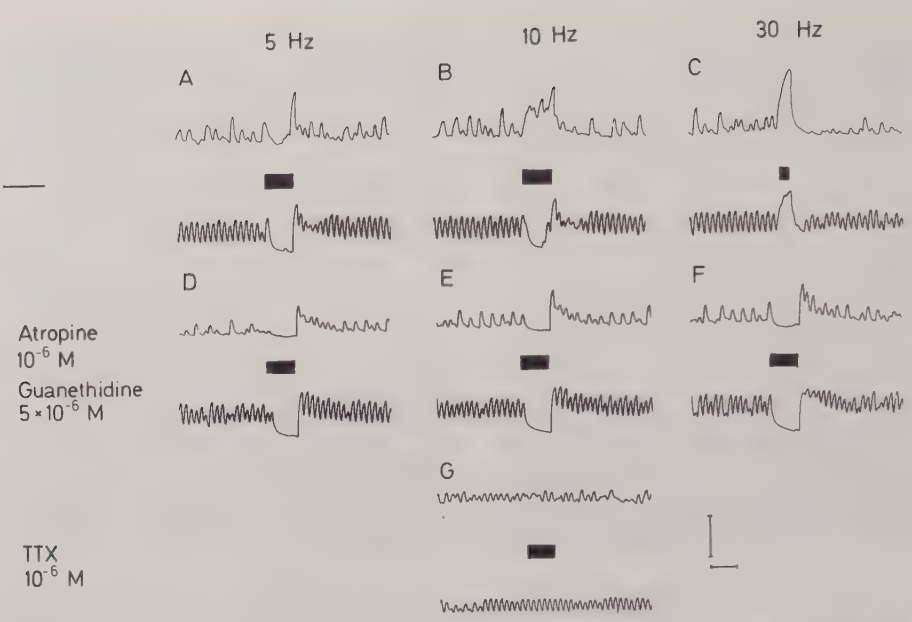

Fig. 6A–G. Spontaneous mechanical activity and mechanical response to electric field stimulation of isolated strips from the longitudinal muscle of pig jejunum in vitro illustrated by a representative experiment. The upper tracing of each pair shows the mechanical activity of the autotransplant and the lower tracing the activity of control jejunum. The registrations in **D, E** and **F** were performed in the presence of atropine (10^{-6} M) and guanethidine (5×10^{-6} M). The muscles in **G** were stimulated in the presence of tetrodotoxin (10^{-6} M). Rectangles between tracings indicate electric field stimulation (1 ms, 8 V/cm). The muscles in **A** and **D** were stimulated with 5 Hz for 30 s, in **B, E** and **G** with 10 Hz for 30 s, in **C** with 30 Hz for 10 s and in **F** with 30 Hz for 30 s. Vertical bar indicates 10 mm. Horizontal bar indicates 30 s

relaxation to nerve stimulation at all frequencies. There was no difference in this respect between specimens from the autotransplants and from intact jejunum. Addition of tetrodotoxin (10^{-6} M) abolished all responses to electrical stimulation indicating that the responses were neurogenic in nature rather than myogenic.

Discussion

The gut is richly supplied not only with adrenergic and cholinergic nerve fibers but also with peptide-containing fibers (cf. Furness and Costa 1980; Schultzberg et al. 1980; Sundler et al. 1980). These latter fibers comprise at least four major types, containing substance P, VIP, enkephalin or somatostatin. Each of the different types of peptide-containing nerve fibers has its own characteristic topographic distribution in the gut wall: Those containing substance P or enkephalin predominate in the myenteric plexus and the smooth muscle (Nilsson et al. 1975a; Pearse and Polak 1975; Alumets et al. 1978). VIP fibers, on the other hand, occur in all layers of the gut wall, but predominantly in the mucosa where they are particularly numerous around the crypts and in the core of the villi (Larsson et al. 1976). In sphincter regions VIP fibers are very numerous in the smooth muscle

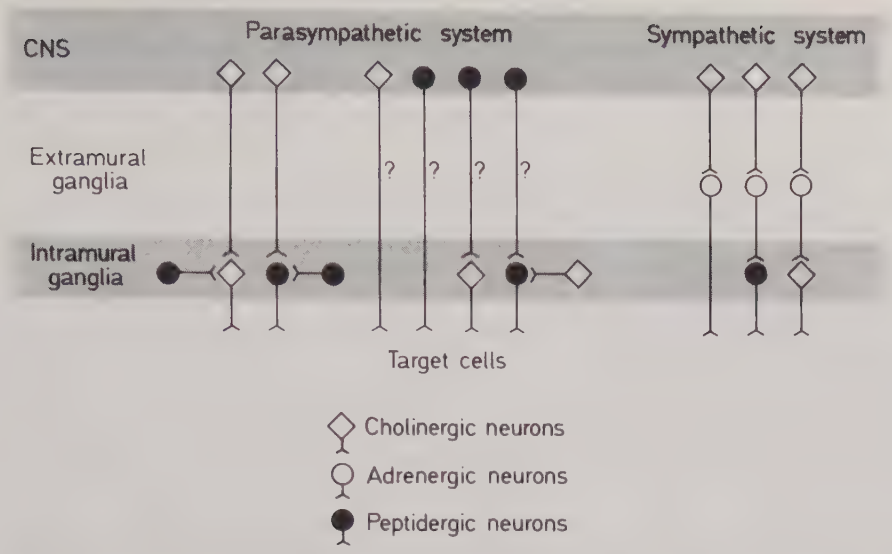

Fig. 7. Schematic representation of the organization and extramural control of the intramural ganglia of the porcine jejunum. The peptidergic neurons are predominantly intramural, some are shown as interneurons. Extramural peptidergic neurons are labeled with question mark, because it has not yet been established if the vagal peptidergic fibers are afferent or efferent. Adrenergic neurons are extramural. Cholinergic neurons are shown as both extramural and intramural; there is no morphological evidence for the existence of extramural cholinergic nerves directly innervating non-neuronal elements, hence the question mark

(Alumets et al. 1979). Somatostatin nerve fibers are found mainly in the myenteric and submucosal plexuses, with only few nerve fibers occurring scattered in the smooth muscle and in the mucosa (Sundler et al. 1980). Adrenergic nerve fibers are found in all layers of the gut (cf. Costa and Gabella 1971; Furness and Costa 1974); they are particularly numerous in the plexuses and around small blood vessels.

All four types of peptide-containing nerve fibers were demonstrated in the porcine jejunum. These nerve fibers may originate from cell bodies located outside or within the gut wall. In addition to bilateral truncal vagotomy, autotransplantation was performed to guarantee complete extrinsic denervation. The peptide-containing nerve fibers had roughly the same frequency and topographic distribution in the autotransplants as in the vagally denervated jejunum and in the innervated jejunum. The unchanged or even slightly elevated concentration of substance P in the wall of the autotransplants as compared to that of the normal jejunum was in good agreement with the immunohistochemical observations. Adrenergic nerve fibers, on the other hand, were lacking in the autotransplants at all times after the operation, indicating that the extrinsic denervation was complete and permanent and that adrenergic nerves do not have an intrinsic origin in the porcine jejunum (see Furness and Costa 1974).

The results of the present study indicate that the major portion of the peptide-containing nerve fibers in the gut is of intramural origin (Fig. 7). This conclusion agrees with the demonstration of various peptide-containing nerve fibers in mouse

small intestine grown in tissue culture (Schultzberg et al. 1978), and with the finding that extrinsic denervation of the guinea pig ileum does not alter the substance P concentration (Franco et al. 1979). However, species differences may exist. Costa et al. (1977) reported that extrinsic denervation lowered the somatostatin concentration by one third in the smooth muscle of the guinea pig ileum. Moreover, in the same species Costa et al. (1980a) found that substance P-containing fibers associated with blood vessels disappeared upon extrinsic denervation, suggesting that they originate outside the intestine; vascular VIP nerves, on the other hand, were of intrinsic origin (Costa et al. 1980b).

Similar to peptide-containing nerves, "cholinergic" nerves, defined by acetylcholinesterase staining, were equally numerous in vagally denervated, transplanted and non-transplanted jejunum. It cannot be excluded that the peptide-containing nerve fibers are included in the very rich population of nerve fibers that can be stained for acetylcholinesterase. However, since acetylcholinesterase staining is a poor marker for cholinergic nerves, it is not possible to distinguish true cholinergic nerves from other types of nerve fibers that may stain for acetylcholinesterase without being cholinergic (Lehman and Fibiger 1979). The existence of true intramural cholinergic nerve fibers in the gut wall is suggested by the fact that in the autotransplants atropine reversed the smooth muscle response to electrical stimulation.

The vagal trunk of the pig is rich in peptide-containing nerve fibers, in particular substance P- and VIP-containing fibers (cf. Lundberg et al. 1979). It is notable that both substance P and VIP, the two predominating neuropeptides in the porcine vagus, accumulated on both sides of the ligature, implying that both VIP and substance P are transported in both directions. There is recent evidence of an axonal transport of substance P predominantly in a centripetal direction within the vagus (Gamse et al. 1979; Brimijoin et al. 1980). It has been suggested that at least part of the substance P fibers in the intestines are afferent (Hökfelt et al. 1975a, b, 1976) and that they reach the central nervous system via the vagus (Gamse et al. 1979). The fact that the substance P levels are unaffected by extrinsic denervation does not support the existence of an extrinsic supply of substance P nerves in the gut. It is possible that the extrinsic supply of substance P-containing fibers is so small that its loss upon extrinsic denervation passes undetected. It is also possible that the lack of nervous input following extrinsic denervation leads to an accumulation of substance P in the intrinsic neurons, thereby masking any loss of substance P-containing nerve endings of extrinsic origin.

The motor activity of the innervated and extrinsically denervated jejunum was studied in vitro. At low stimulation frequencies the smooth muscle of the innervated jejunum responded by a relaxation followed by contraction upon cessation of stimulation. With increasing stimulation frequencies the duration of the relaxation decreased; at high frequency stimulation there was no relaxation, only a contraction. In the extrinsically denervated jejunum low frequency stimulation induced no or only a weak relaxation; high frequency stimulation induced contraction. After cholinergic and adrenergic blockade the muscle responded by relaxation at all frequencies; the response was similar in innervated and denervated specimens. Hence, the results suggest the involvement of intramural non-cholinergic, non-adrenergic nervous mechanisms in the regulation of smooth

muscle activity in the small intestine. It appears likely that the peptide-containing nerve fibers are responsible, and it is not unlikely that the neuropeptides act as transmitters. On the whole, extrinsic denervation had only minor effects on the motor activity of the porcine jejunum, possibly reflecting the high degree of autonomy of the gut.

In conclusion, acetylcholinesterase-positive nerve fibers as well as peptide-containing nerve fibers are intrinsic to the porcine gut wall, while adrenergic nerve fibers are derived from ganglia located outside the gut wall. The peptide-containing nerve fibers of the vagus do not appear to provide a quantitatively significant contribution to the nerve supply of the gut wall. The peptide-containing nerve fibers are probably involved in the non-cholinergic, non-adrenergic control of smooth muscle activity.

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JEJUNAL AUTOTRANSPLANTATION: EFFECTS ON MUCOSAL ARCHITECTURE AND ENDOCRINE CELLS

An experimental study in the piglet

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The intestinal mucosa harbours a great number of biologically active peptides. Some of these peptides are produced in endocrine cells, others in neuronal elements, and still others in both endocrine cells and neurons (cf CREUTZFELDT 1980). Using jejunal autotransplants we have previously demonstrated that various peptide-containing neurons are intrinsic to the porcine gut wall, i.e. they originate from cell bodies in the submucosal and/or myenteric plexuses (MALMFORS et al 1980, 1981).

Intestinal autotransplants have been used experimentally and clinically to repair the defect after resection of the cervical oesophagus (SEIDENBERG et al 1959, OKMIAN 1976, cf GERMAIN et al 1980). Previous reports regarding the effects of autotransplantation on the mucosal morphology are controversial; some claim no change, (LILLEHEI et al 1962, NAKAYAMA et al 1964, ALICAN et al 1976, UEMICHI et al 1977, GERMAIN et al 1980) while others report mucosal flattening (BALLINGER et al 1962, PULL TER GUNNE 1975). To the best of our knowledge the effect of autotransplantation on the various endocrine cell populations has not previously been studied.

The aim of the present study was to investigate the effects of autotransplantation on the intestinal mucosa with special emphasis on its endocrine cells. It was attempted to distinguish between the effects of external denervation on one hand and exclusion from intestinal continuity on the other.

MATERIAL AND METHODS

The series consisted of 17 piglets weighing between 4.4 and 8.4 kg (mean value 6.7 kg). Thirteen other operated piglets were excluded from the study because of necrotic transplants or technical failures.

Operative technique

Autotransplantation of a jejunal segment was performed in three different ways:

I. Autotransplantation with an ischaemic period and with exclusion from intestinal continuity.

II. Autotransplantation without ischaemia but with exclusion from intestinal continuity.

III. Autotransplantation without ischaemia and with transplant in intestinal continuity.

The use of these different procedures gave an opportunity to distinguish between the effects of extrinsic denervation, of exclusion from intestinal continuity, and of ischaemia.

In all operations endotracheal anaesthesia with 50% O_2 and 50% N_2O was used. In addition, Ketamine hydrochloride (Ketaminum NFN), Droperidole (Droperidolum INN) and Alcuronium chloride (Alcuronum NFN) were used. The operations were performed under sterile conditions with microsurgical and pediatric surgical instruments.

I. Autotransplantation with an ischaemic period and with exclusion from intestinal continuity

Nine animals were operated. Laparotomy was performed in the midline. A segment, 10 - 20 cm, of the proximal jejunum was resected to be used as an autotransplant. The artery and vein of the transplant, measuring 1.5 and 2 - 3 mm in diameter respectively, were not divided until intestinal continuity had been restored with an end-to-end suture. The adjacent segments of the jejunum became ischaemic and had to be resected. Immediately after the vascular pedicle was divided, infusion into the artery was started with refrigerated Perfadex^R solution with heparin 5 IE/ml. The aorta and the inferior caval vein were dissected free several cm cranially from their bifurcations. The transplant vein was anastomosed end-to-side to the caval vein with microsurgical technique. An end-to-side microanastomosis between the transplant artery and the aorta was performed after a suitable opening in the aortic wall had been made. After revascularization, the proximal end of the transplant was closed and the distal end was drawn through and sutured to the abdominal wall as a jejunostomy.

II. Autotransplantation without ischaemia but with exclusion from intestinal continuity

Three animals were operated. Suitable mesenterial vessels were identified. The width of the artery was approximately 1.5 mm and of the vein 2 - 3 mm. First, the vein was divided between vascular clamps

and immediately resutured with microsurgical technique. The artery was then divided and resutured in the same way (Fig. 1 A). After this, a wedge-resection of a 10 - 20 cm segment of jejunum was performed. During vascular clamping the circulation of the transplant was maintained via arcade vessels. Thus, the autotransplant obtained had not been ischaemic and was already revascularized (Fig. 1 B). Intestinal continuity was restored and the transplant was sewn to the abdominal wall as a jejunostomy.

III. Autotransplantation without ischaemia and with transplant in intestinal continuity

Five animals were used. An autotransplant was obtained in the same way as in II. The transplant was interposed into intestinal continuity with two end-to-end anastomoses (Fig. 1 C).

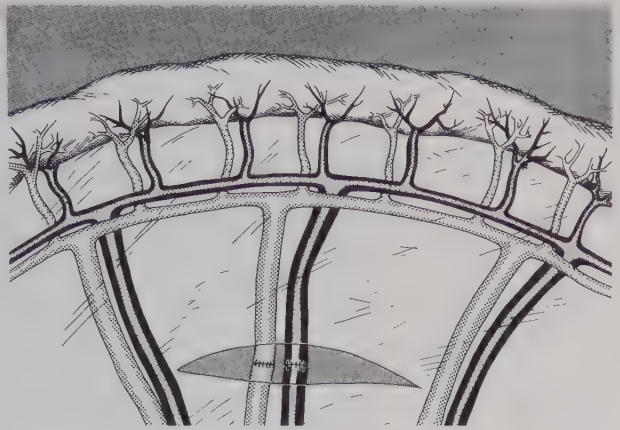
The piglets in the first group were sacrificed after 3 to 17 weeks. The piglets in groups II and III were sacrificed after 6 to 8 weeks. At sacrifice the autotransplant was dissected free while the piglet was under anaesthesia. Specimens were taken from the autotransplant and from the normal jejunum close to the intestinal anastomosis.

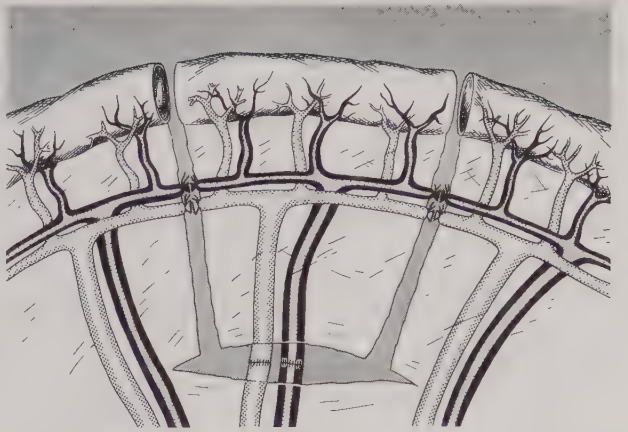
Tissue processing

Specimens from the autotransplants and from jejunum in situ were immediately frozen to the temperature of liquid nitrogen in a mixture of propane and propylene. After freeze-drying, the specimens were fixed by exposure to gaseous formaldehyde for 1 hr at 80°C or to gaseous diethylpyrocarbonate for 3 hrs at 55°C, and embedded in paraffin. Sections were cut at 6 µm.

Immunocytochemical procedures

Sections were deparaffinized in xylene, hydrated in graded ethanol solutions and processed for the immunocytochemical demonstration of a variety of gut peptide hormones. The antiserum was applied for 17 hrs at 4°C. Details on the antisera used are given in Table 1. All the peptide antisera have previously been used in immunocytochemistry (ceg SUNDLER et al 1979). The site of the antigen-antibody reaction in the tissue sections was revealed by the peroxidase-antiperoxidase (PAP) technique of STERNBERGER (1974). All solutions contained 0.25%





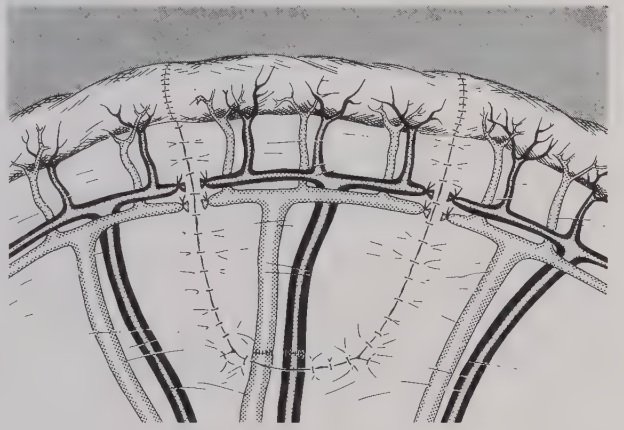


Fig. 1 A. - C. Autotransplantation of a jejunal segment without producing ischaemia.

A. (Upper left). Vessels are divided and re-sutured. Sufficient collateral circulation.

B. (Upper right). Wedge-shaped resection creates an autotransplant that is already revascularized.

C. (Left below). Intestinal continuity is restored.

Table 1. Source and working dilutions of peptide antisera

Antisera against	Antigen	Working dilution	Code	Source
Enkephalin	Synthetic leu-enkephalin	1:240	"leu-enk"	R.J. Miller, Wellcome Res. Labs. Borroughs Wellcome Co. Triangle Park N.C. USA
CCK	Pure porcine CCK-39	1:640		W. Schlegel, Dept. of Gynecology Univ. of Münster, Germany
Neurotensin	Synthetic bovine neurotensin	1:640	HC-8	R. Carraway, Harvard Med. Sch. Boston, MA. USA
Somatostatin	Synthetic bovine somatostatin	1:2560	19 578	M.P. Dubois, Inst. Nat. Rech. Agr. Nouzilly, France
Secretin	Pure porcine secretion	1:2560	5585	O. Schaffalitzly, de Muckadell Bispebjerg Hosp., Copenhagen Denmark
Motilin	Pure porcine motilin	1:640	MBR 01-6	M. Yanaihara, Shizuoka Coll. of Pharmacy, Shizuoka, Japan
GIP	Pure porcine GIP	1:5120	R-DH/19/77	T. O´Dorisio, Ohio State Univ. Ohio, USA

human serum albumin and 0.25% Triton X-100. The sections were rinsed and mounted in Permount^{R}. Conventional staining controls included deletion of the first antiserum, deletion of the second antiserum and deletion of both antisera. As specificity controls served sections incubated with peptide antiserum inactivated by the addition of antigen in excess (10 - 100 µg of pure natural or synthetic peptide per ml diluted antiserum). Unlabelled goat anti-rabbit IgG (used in dilution 1:20) was obtained from SBL, Stockholm, Sweden and PAP complex (diluted 1:320) was purchased from Dakopatts, Copenhagen, Denmark.

Quantitative histologic analysis

Light microscopic morphometry of the mucosa

Sections were cut perpendicular to the mucosal surface and stained with haematoxylin and eosin. The total thickness of the mucosa, the height of the villi and the depth of the crypts were measured at 125 x magnification (objective 10 x and eye piece 12.5 x). In addition, the number of crypts per unit length was counted. The height of the villi and the depth of the crypts were expressed as percentage of the total mucosal thickness.

Cell counts

Immunostained sections cut perpendicular to the mucosal surface were used. The number of each of the endocrine cell types was counted in 4 - 11 randomly selected visual fields covering the whole mucosa (125 x magnification) from each animal.

Statistical analysis

Transplants were compared with jejunum in situ using Student´s t-test for paired observations.

RESULTS

Mucosal architecture

The mucosa was flattened in transplants excluded from intestinal continuity (I, II). The change (approx. 20%) was statistically significant. The thickness of the mucosa was unchanged in transplants in intestinal continuity (III) (Table 2, Fig. 2).

Table 2. Mucosal thickness / Relative height of villi (Mean ± S.D.)

Op. procedure	I	II	III
Transplant	10.4**±1.9 / 40.1±5.7	8.0*±0.0 / 44.3±2.9	9.9±3.6 / 44.0±4.3
Jejunum in situ	12.9 ± 1.1 / 49.6 9.5	10.7 ±0.5 / 41.3±2.1	10.6±2.2 / 42.0±4.7
	$p<0.01$, n=8	$p<0.05$, n=3	n=5

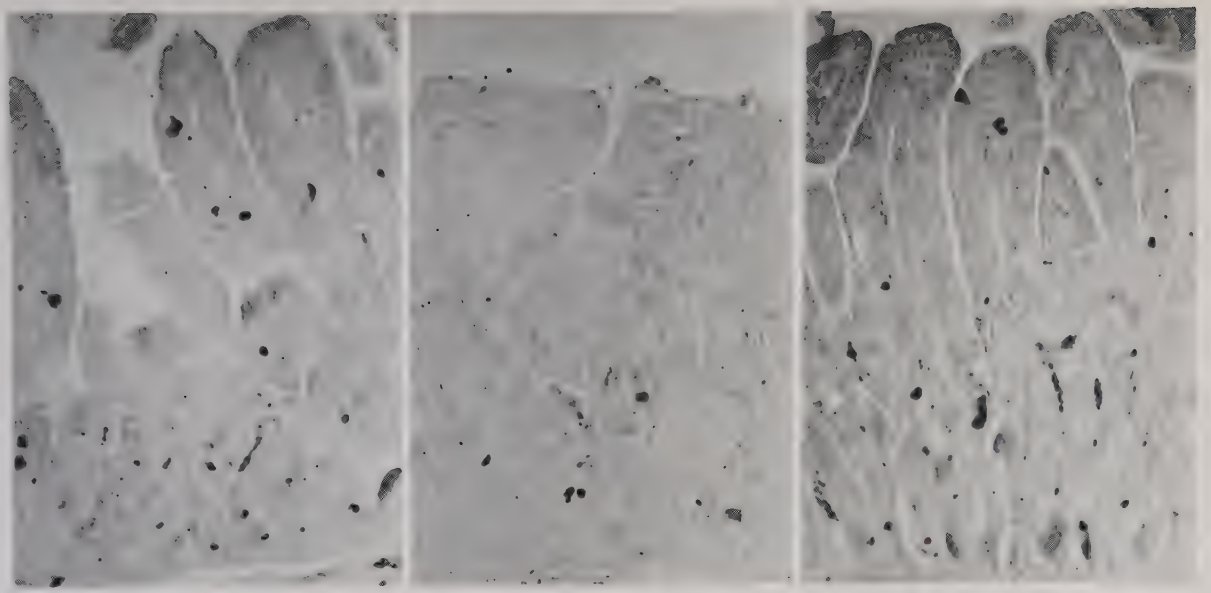


Fig. 2. Porcine jejunum. Sections processed for the immunocytochemical demonstration of somatostatin using immunoperoxidase technique. Left: jejunum in situ. Numerous somatostatin cells randomly distributed in the mucosa. Middle: autotransplant excluded from intestinal continuity. Marked reduction in the number of somatostatin immunoreactive cells. Note also flattening of the mucosa. Right: autotransplant re-inserted in intestinal continuity. Somatostatin cells are as numerous as in the jejunum in situ. Normal mucosal architecture. (x 200).

The relative height of the villi (per cent of total mucosal thickness) was unchanged in all transplants (I, II, III) (Table 2). The villi of the autotransplants were often slightly club-shaped.

The number of crypts per visual field was unaffected by all three operative procedures (Table 3).

Endocrine cells

The porcine jejunum harboured a great variety of peptide hormone producing cells. Enkephalin, GIP, somatostatin and CCK immunoreactive cells were fairly numerous. Neurotensin cells, secretin cells and motilin cells were few. Enkephalin cells, somatostatin cells and motilin cells predominated in the crypts, CCK cells, GIP cells and neurotensin cells were randomly distributed in the mucosa, while the secretin cells occurred almost exclusively on the villi.

Autotransplantation with exclusion from intestinal continuity (I, II) resulted in a reduction in the number of certain endocrine cells. Ischaemia did not seem to affect this reduction. Thus, the number of enkephalin cells was reduced by about 40%. The corresponding reduction for CCK and somatostatin cells were 40% and 30% respectively. GIP cells were not significantly reduced in number. The neurotensin, secretin and motilin cells were too few to allow reliable counting. Autotransplants in intestinal continuity (III) did not differ from jejunum in situ with respect to the number of endocrine cells (Table 4, Fig. 2).

Both the mucosal architecture and the distribution of endocrine cells seemed to be unaffected by the time after the operation and by the length of the ischaemic period.

DISCUSSION

Transplantation is the only operative procedure that guarantees a total extrinsic denervation of an intestinal segment. In addition it results in a complete interruption of the lymphatic system. The lymphatics are known to regenerate completely within a few weeks (GOOTT et al 1960). The follow up time was chosen to ensure lymphatic regeneration.

Table 3. Number of crypts per unit length

Op. procedure	I + II	III
Transplant	12.8 ± 1.7	14.3 ± 1.1
Jejunum in situ	12.8 ± 2.0	13.8 ± 1.0
Mean ± S.D.	n = 5	n = 5

Table 4. Number of endocrine cells (Mean ± S.D.)

	Enkephalin	CCK	GIP	Somatostatin
Transplant in intestinal continuity	12.1 ± 3.8	5.9 ± 2.4	9.2 ± 2.2	8.0 ± 1.0
Jejunum in situ	11.5 ± 3.9	5.9 ± 1.6	8.7 ± 2.1	7.7 ± 1.7
n = 5				
Transplant excluded from intestinal continuity	5.2* ± 2.0	4.5* ± 1.9	5.2 ± 1.8	4.8* ± 3.1
Jejunum in situ	9.8 ± 3.9	7.7 ± 3.1	6.3 ± 1.6	7.2 ± 4.0
n = 6	$p<0.05$	$p<0.05$		$p<0.05$

BALLINGER et al (1962) found mucosal changes after autotransplantation in dogs and suggested that these were caused by the extrinsic denervation per se. The changes consisted in an initial total loss of villi, regenerating within a couple of weeks but remaining short and club-like. Also PULL TER GUNNE (1975) described a shortening of the villi after ileal autotransplantation in dogs.

LILLEHEI et al (1962), on the other hand, found no mucosal changes in their pioneering autotransplantation experiments in dogs. ALICAN et al (1971) also found normal mucosa at different times after intestinal autotransplantation in dogs. In addition, there are several reports claiming normal mucosal morphology following intestinal autotransplantation in man (NAKAYAMA et al 1964, UEMICHI et al 1977, GERMAIN et al 1980). None of these studies include quantitative histologic analysis.

STAUFFER (1975) reported that exclusion from intestinal continuity alone did not affect the mucosal morphology in mini-pigs. The results of the present study suggest that extrinsic denervation per se does not affect the mucosal thickness. Extrinsic denervation combined with exclusion from the intestinal continuity moderately lowered the mucosal thickness without affecting the relative height of the villi. It seems possible that the combination of extrinsic denervation and intestinal exclusion is responsible for the flattening of the mucosa.

The porcine jejunum is rich in endocrine cells. Generally, the spectrum of peptide hormones and the frequency of occurrence of their cells of origin corresponds to that of other mammals, except for the enkephalin immunoreactive cells which are particularly numerous in the porcine small intestine, while being scarce or absent from this location in other species (ALUMETS et al 1979). Autotransplantation of a jejunal segment with exclusion from intestinal continuity resulted in a marked reduction in the frequency of occurrence of several endocrine cell types. The reduction was, however, not uniform in that the enkephalin and CCK cells decreased by about 40%, the somatostatin cells by 30%, and the GIP cells by 20% (statistically not significant). The reason for this varying effect on the individual endocrine cell types is not known. The fact that autotransplantation with reestablishment of in-

testinal continuity did not overtly affect the number of endocrine cells indicates that extrinsic denervation per se did not produce the changes. Rather, exclusion from intestinal continuity was responsible. Conceivably, the passage of food is necessary for maintaining the full morphological integrity of the mucosa including its endocrine cells.

In conclusion - extrinsic denervation per se does not seem to affect the endocrine cells in the jejunum. In this respect the endocrine cells behave much in the same way as the peptide-containing neuronal systems of the gut wall, which also seem to be unaffected by extrinsic denervation (MALMFORS et al 1980, 1981). Exclusion from intestinal continuity on the other hand causes a marked reduction in the number of various endocrine cells, probably because they are deprived of the stimulating action of the intestinal content.

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